$$R_2S^{\bullet+} + A^{\bullet-} \rightarrow R_2S + {}^1A^* \quad (1a)$$

$$^1A^* \rightarrow A + h\nu \quad (1b)$$

dependence (constant microwave frequency) of the observed fluorescence intensity yields the superimposed EPR spectra of the positive and negative ions.[17] The FDMR experiment has been described in detail elsewhere.[18,19]

The spectrum in Figure 1a consists of an intense feature due to the unresolved EPR lines of the anthracene radical anion and the five-line EPR spectrum of the thietane radical cation. At higher thietane concentrations and higher temperature, the monomer sulfide radical cation spectrum disappears and is replaced by the nine-line EPR spectrum (outer lines not visible) of the thietane dimer radical cation (Figure 1b). In similar experiments carried out in alkane solvents (e.g., *n*-hexane), monomer sulfide radical cations were not detected; only dimer radical cations were detected.[20]

The greater stability (with respect to dimer formation and/or deprotonation) of monomer thioether radical cations in aromatic solvents versus alkane solvents can be accounted for by complex formation in the former case. The charge-transfer interaction with the aromatic solvent can be inferred from the EPR parameters, which are significantly shifted from the values measured in Freon matrices. Table I lists the hyperfine coupling constants for the radical cations of thietane, tetrahydrothiophene, and dimethyl sulfide measured by FDMR in toluene and by EPR in Freon matrices. In every case the hyperfine coupling constant is ~30% smaller in toluene. This is not a temperature effect or a matrix effect. Transfer of spin from sulfur to toluene accounts for the smaller coupling to the β-protons on the sulfide and should lead to a concomitant reduction in the *g* factor, which is also observed.

When *m*-xylene is the solvent, the monomer radical cation coupling constants are between 10 and 15% smaller than in toluene and the *g* factors also undergo a further reduction. This indicates an even greater degree of charge transfer in *m*-xylene and correlates with the relative gas phase ionization potentials of toluene (8.8 eV) and *m*-xylene (8.5 eV). The EPR parameters of dimer sulfide radical cations are not nearly as solvent dependent, which indicates that the solvent interaction is broken when the unpaired electron is coordinated instead to the free p-electron pair of another sulfur atom. Furthermore, the liquid-phase EPR parameters of the dimer radical cations are in good agreement with those measured in Freon matrices,[7] which shows the absence of any matrix effect.

Binding of chlorine atoms by benzene and substituted benzenes changes their reactivity. The arene–chlorine atom complex is a much more selective reagent (shows a greater preference for abstracting tertiary versus primary H atoms) than free chlorine atoms.[11-15] Likewise our results indicate that complexation of thioether radical cations in aromatic solvents decreases their reactivity. Solvent effects are very important factors in determining the reactivity of a wide variety of reactive radical intermediates and can be used to great advantage for achieving greater chemical selectivity.

In summary, we have made the first liquid-phase observation of thioether monomer radical cations by using time-resolved FDMR spectroscopy. The detection of the thioether radical cations was possible because of the formation of a unique radical cation–arene π-molecular complex in aromatic solvents involving the interaction of the predominantly 3p SOMO of the thioether radical cations with the arene π-systems and also because of the time-resolved capability of FDMR. FDMR detects radical ions on the 10^{-8}–10^{-6} s time scale and thus does not determine their long-term (>10^{-6} s) stability. The thioether radical cation–arene complexes should possess charge-transfer bands in the visible or near-UV by analogy with the arene–chlorine atom complexes [21-23] and thus could be studied by optical methods as well.

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Temperature of Polypeptide Inverse Temperature Transition Depends on Mean Residue Hydrophobicity

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For the many hydrophobicity scales for amino acid residues in protein and polypeptides,[1-11] there is reasonable agreement for a sequence in which Phe (F) > Ile (I) ≃ Leu (L) > Val (V) > Ala (A) > Gly (G). When these residues are substituted at position 4 within $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$, abbreviated poly(VPGVG), to give a structure written poly[f_X(VPGXG),f_V(VPGVG)] where f_V is the mole fraction of pentamers with valyl residues at position 4 and f_X is the mole fraction of pentamers with a guest residue, X, at position 4 with $f_X + f_V = 1$, the temperature of a reversible aggregational transition in water (actually a phase transition described as a coacervation) is here demonstrated to be inversely dependent on the mean hydrophobicity, and in addition, the heat of the transition is found to be directly proportional to the mean residue hydrophobicity in studies where the temperature and heat of the transition were determined by differential scanning calorimetry (DSC). With the use of transition temperature to determine relative hydrophobicity having been established in this molecular system, data for Trp (W), Tyr (Y), and Met (M) complete the values for residues comprising the apolar half of the natural amino acids in this first hydrophobicity scale based on a physical property so integral to the process of protein folding and assembly. Importantly, the transition temperature, in this relatively simple polypeptide (pro-

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(1) Prior to the present report, relative hydrophobicities were derived from relevant but indirect approaches. For example, the Nozaki and Tanford scale was based on relative solubilities of amino acids in organic solvents;[2] the Bull and Breese scale utilized surface tension of amino acid solutions;[3] Hopp and Woods used the correlation of hydrophilicity with antigenic determinants;[4] a number of scales derive from the distribution of residues buried within or on the surface of globular proteins;[5-10] and more recently and more closely related to the present report, the physically relevant partial molar heat capacities of peptide moieties and amino acid side chain equivalents have been used but with limitations due to low solubility of the side chains of the Ala, Val, Leu, and Ile amino acids and due to the assumed additivity of polar and apolar components.[11]
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tein-based polymer) system, which is nonetheless capable, when suitably substituted and cross-linked, of efficient thermomechanical and chemomechanical free energy transductions,[12] can now be used to study the effect of various perturbations on the expression by hydrophobicity, such as polar residues, salts in solution, isotope effects (e.g., ^2H_2O), organic solvents, etc.

Repeat peptides of elastin such as $(VPGVG)_n$ and the related $(VAPGVG)_n$ exhibit an increase in order both intramolecularly and intermolecularly when the temperature is raised through a critical temperature range, that is, they exhibit inverse temperature transitions.[13] This is most unambiguously demonstrated by cyclo$(VAPGVG)_2$, which reversibly crystallizes when the temperature is raised and dissolves when the temperature is lowered through a transition range.[14] Another informative cyclic analogue which aggregates when the temperature is raised is cyclo$(VPGVG)_3$. The crystal structure of this analogue is notable (1) due to a substantial water content, with the water not occurring between molecules but rather within the cylindrical stack of cyclic molecules; (2) due to the extensive hydrophobic interactions between molecules, within and between stacks;[15] and (3) due to the conformation of the cyclic molecule being a cyclic conformational correlate[16] of linear poly(VPGVG), which itself self-assembles, when the temperature is raised, into fibers comprising fibrils which are bundles of parallel aligned twisted filaments formed from the supercoiling of loose hydrophobically assembled helical structures of poly(VPGVG) called β-spirals.[13,17] A wide range of physical methods have been used to characterize the increase in order intra- and intermolecularly as the temperature is raised through the folding and aggregational transition.[18] Now that an increase in polypentapeptide order increasing temperature is established, it is natural to look to ordered water molecules becoming disordered to achieve the net entropy increase when the temperature is raised through the transition in keeping with the second law of thermodynamics. Furthermore, since the secondary structure of poly(VPGVG) does not change on passing through the transition,[19] it becomes obligatory that waters of hydrophobic hydration be considered.

One direct check of this rationale is to synthesize a more hydrophobic analogue in which the conformation is the same as that of poly(VPGVG) before the transition and changes to the same folded conformation as that of poly(VPGVG) after the transition. This has been achieved with the poly(IPGVG) which has an added CH_2 moiety per pentamer but which retains important β-branching at position 1.[20,21] The result is that the increase in hydrophobicity lowers the temperature of the transition and increases the heat of the transition.[22] The interpretation is that the increase in endothermic heat required to drive the transition reflects the energy required to destructure the greater numbers of waters of hydrophobic hydration.

Because β-branching provides an important hydrophobic interaction during folding, i.e., a $Val_1^1\gamma CH_3...Pro_1^2\delta CH_2$ interaction identified by the nuclear Overhauser effect,[21,23] this position does not lend itself to many isomorphic substitutions, but position 4 does. [For example, poly(APGVG), rather than reversibly forming a viscoelastic coacervate, irreversibly forms a granular precipitate when the temperature is raised.][24] The general formula poly-$[f_X(VPGXG),f_V(VPGVG)]$ provides a series of polypentapeptides of varying hydrophobicity in which the temperature of the transition and the heat of the transition could provide a hydrophobicity scale, a scale derived directly from hydrophobically driven folding and assembly of interest to protein structure and function.

Reported here are DSC data on 19 polypentapeptides of molecular weight greater than 50 000 Da. The synthesis and verification of these 19 polymers will be presented elsewhere; they constitute a fraction of more than 200 high-polymer syntheses that provide the necessary knowledge base for confidence in the results of Figures 1 and 2. Representative DSC data for three compositions are given in Figure 1, where the transition temperature is defined as T_b from the extremum of the derivative curve. The heats of the transition were determined as previously described.[22] Data for the 19 polypentapeptides are plotted in

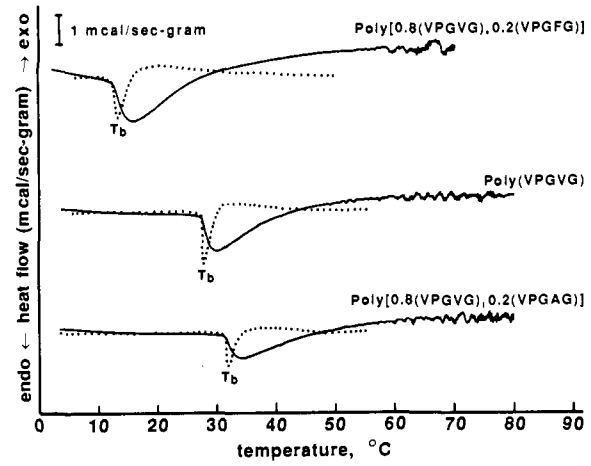

Figure 1. DSC thermograms (solid line) and their derivatives with respect to temperature (dotted line) of three polypentapeptides in H_2O. The scan rate was 0.23 °C/min. The peptide concentration is 40 mg/mL.

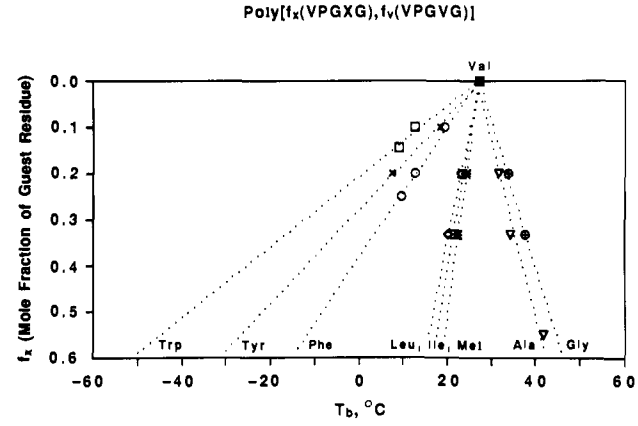

Figure 2. The dependence on mole fraction of guest residue, f_X, of the temperature (T_b) of an inverse temperature transition, given as the extremum on the derivative with respect to temperature of the DSC scan for 19 different polypentapeptides with the general composition, poly-$[f_X(VPGXG),f_V(VPGVG)]$. The dependence of f_X is seen to be very nearly linear such that the values may be extrapolated to a reference value for f_X to give a comparison of hydrophobicities.

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Figure 2 as the mole fraction of guest residue, f_X, versus T_b. Compositions with values of T_b below 0 °C cannot be studied because of inability to achieve the dissolved state below the freezing point of water.

From this data the relative hydrophobicities are seen to be Trp > Tyr > Phe > Leu ≃ Ile ≃ Met > Val > Ala > Gly. While the accuracy with which the experimental heat (ΔH) of the transition can be determined is less than that of the temperature, a similar scale is obtained from the difference in heats due to the substitution, i.e., $\delta\Delta H(X) = \Delta H(VPGXG) - \Delta H(VPGGG)$ with extrapolation to $f_X = 1$. The results of the $\delta\Delta H$ in kilocalories/mole are W (6.2) > F (5.6) > Y (4.6) > L (3.9) > I (3.6) > V (2.0) > M (1.8) > A (0.4) > G (0.0). Clearly, Trp comes out to be the most hydrophobic in this functional scale, and interestingly there is an interchange between the Tyr and Phe order when temperatures and heats of the transition are compared. It may ultimately be more appropriate to use an entropy scale, i.e., $\delta(\Delta H/T)$. From the practical side, when the temperature scale is used, it becomes possible to choose a combination of residues that gives any desired value for T_b from ~0 °C to about 60 °C. This sets the stage for the proper consideration of polar groups including the demonstration that COO^- is very much more polar than COOH[12,25,26] and allows that the temperature and heat of the transition may be used to determine the effects of other perturbations, such as NaCl and other salts, urea, guanidine hydrochloride, ethylene glycol and other alcohols, dimethyl sulfoxide, etc., on the expression of hydrophobicity.

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2D NMR Assignment of Hyperfine-Shifted Resonances in Strongly Paramagnetic Metalloproteins: Resting-State Horseradish Peroxidase

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Two-dimensional (2D)[1] NMR of small diamagnetic biomolecules is now routinely used to both assign ^1H resonances and determine structure. To date, these 2D methods have found much less application to paramagnetic metalloproteins in the belief that the rapid paramagnetic-induced relaxation would render cross peaks undetectable. For bond correlation or COSY data, the broad lines (short T_2's) result in both rapid decay of coherence and extensive cancellation of antiphase cross peaks,[2,3] while the short T_1's severely short-circuit the buildup of nuclear Overhauser effect or NOESY cross peaks.[3,4] Numerous paramagnetic metalloproteins yield remarkably well resolved ^1H NMR spectra,[5,6] for which the extraction of the significant information content of the hyperfine shifts has been thwarted by the lack of an effective assignment methodology. We have shown recently that the 1D NOE is surprisingly effective in obtaining crucial but limited assignments in both weakly[7,8] and strongly paramagnetic proteins.[9-11] The vast improvement of the information content of 2D relative to 1D NMR of small, weakly paramagnetic, low-spin ferric hemoproteins with narrow lines has been demonstrated.[12,13] We have shown[14] recently that, while paramagnetism will always diminish NOEs relative to a diamagnetic system, the paramagnetic influence is increasingly "suppressed" as the size of the protein increases. Because of the large number of paramagnetic metalloproteins that would benefit from the demonstration of effective 2D methodology, we investigate here the utility of NOESY and COSY on a moderately sized enzyme (42-kDa horseradish per-

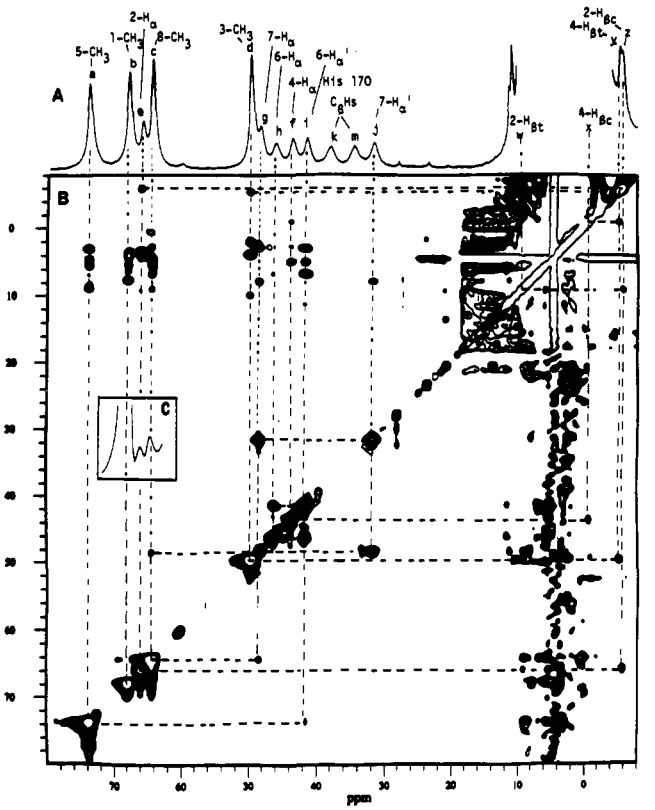

Figure 1. (A) ^1H NMR (300 MHz) reference trace for 3 mM HRP in a 5-mm tube in ^2H_2O, pH 7.0 at 55 °C, with peaks labeled a-m and w-z as well as with previous assignments.[10,11] (B) NOESY map collected with 10-ms mixing time, with labeled cross peaks. The inset (C) gives a slice in the f_1 direction through the 1-CH_3 on the diagonal which yields a weak cross peak to 8-CH_3.

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