

## Macromolecular solvation energies derived from small molecule crystal morphology

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### Abstract

The morphology of small molecule crystals provides a model for evaluating surface solvation energies in a system with similar packing density to that observed for amino acid residues in proteins. The solvation energies associated with the transfer of methylene and carboxyl groups between vacuum and aqueous phases are estimated to be approx. +40 and -260 cal/Å<sup>2</sup>, respectively, from an analysis of the morphology of succinic acid crystals. These solvation energies predict values for contact angles in reasonable agreement with measurements determined from macroscopic monolayer surfaces. Transfer free energies between vapor and water phases for a series of carboxylic acids are also predicted reasonably well by these solvation energies, provided the surface exposure of different groups is quantitated with the molecular surface area rather than the more traditional accessible surface area. In general, molecular surfaces and molecular surface areas are seen to have important advantages for characterizing the structure and energetics of macromolecular surfaces. Crystal faces of succinic acid with the lowest surface energies in aqueous solution are characteristically smooth. Increasing surface roughness and apolarity are associated with higher surface energies, which suggests an approach for modifying the surface properties of proteins and other macromolecules.

**Keywords:** crystal morphology; succinic acid; solvation energies

Interactions between water and macromolecules contribute significantly to the energetics of protein folding, stability, and function. Although the importance of these solvation, or hydrophobic, interactions between water and macromolecules has been widely appreciated since Kauzmann's 1959 review, the energetic details of these interactions are complex and rather poorly understood. Although theoretical treatments are available (Pratt & Chandler, 1986), most studies have focused on empirical approaches for estimating the energetics of hydrophobic interactions. These estimates are typically derived from measurements of the free energy of transfer,  $\Delta G_H$ , of amino acid side chains (or analogues) from nonpolar to aqueous solutions (Nozaki & Tanford, 1971; Hine & Mookerjee, 1975). Atom-based potential functions (Eisenberg & McLachlan, 1986; Ooi et al., 1987) have been developed by parameterizing these transfer free energies with the expression:

$$\Delta G_H = \sum_{i=1}^N \text{area}_i \Delta \sigma_i, \quad (1)$$

where  $\text{area}_i$  is the exposed surface area of the  $i$ th atom being transferred from a nonpolar to a polar medium, and  $\Delta \sigma_i$  is the change in surface free energy of the  $i$ th atom. Depending on the formulation,  $\Delta \sigma_i$  may be constant for all atom types or may be parameterized according to the polarity of different atom types. The summation is taken over all  $N$  atoms in the group or molecules transferred between the nonpolar and aqueous phases.

Following Richards (1977), macromolecular surfaces may be defined by rolling a spherical probe molecule around the van der Waals surface of the macromolecule. In most applications, the surface area used in evaluating Equation 1 is the accessible surface area (ASA). As defined by Lee and Richards (1971), the ASA is determined from the area of the surface traced by the center of a probe sphere as it is rolled over the van der Waals surface of a macromolecule. Consequently, the accessible surface is displaced from the van der Waals surface of a macromolecule by the radius of the probe sphere. An alternative, less frequently used definition for the surface area,

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the molecular surface area (MSA), may be calculated from the area of a continuous envelope stretched over the van der Waals surface of a macromolecule. This continuous envelope is described by the position of the inner surface of the probe sphere as the probe moves in contact with the van der Waals surface. The energetic consequences of using either ASAs or MSAs to estimate  $\Delta\sigma$  for various functional groups by fitting these parameters to transfer measurements with Equation 1 tends to be minimal because the ASAs and MSAs of the various groups are usually proportional (Chothia, 1984), and the relative values of  $\Delta\sigma$  are unaffected by the choice of area.

Although descriptions of solvation energetics based on transfer free energies have been quite useful, there are several potential problems in applying these measurements to macromolecules: (1) The concentrations of amino acids in transfer experiments are 1–3 orders of magnitude more dilute than the side-chain concentrations found in proteins (estimated to be about 10 M, based on typical protein densities and amino acids weights). Concentration-dependent effects are expected to have a significant influence at these higher concentrations because the behavior of 10 M aqueous solutions may be strongly nonideal. (2) The surface area measurement relevant to transfer experiments (such as ASA, MSA, or some other definition) has not been established rigorously, although ASAs are used traditionally because they provide a measure of the number of water molecules that can be packed around a surface. (3) Many methods for estimating solvation energies assume that these energies are proportional only to the ASAs of molecules. The influences of differing solute and solvent molecular volumes, surface curvature, and surface roughness can significantly perturb the energetics of hydrophobic interactions, as has been described recently (Nichols et al., 1991; Sharp et al., 1991a,b). These factors complicate a quantitative description of solvation energies for macromolecules.

The morphology of small molecule crystals provides a model system for evaluating solvation energies under conditions more representative of those found in proteins. Gibbs (1928) demonstrated that the equilibrium morphology of a crystal will minimize the surface free energy. The surface free energy reflects the differences in energies between the solute–solute and solvent–solvent interactions and the solute–solvent interactions at the crystal surface. This latter quantity is precisely the interaction energy required for a quantitative description of solvation effects in proteins. The packing density of small molecule crystals is similar to that found in proteins, so that estimates of the surface free energies from small molecule crystals should be more representative of the types of interactions at the protein–water interface than those obtained from transfer free energy measurements. Furthermore, the structure dependence of the surface free energy may be addressed from the structures (derived from diffraction analyses) of the small molecule crystal faces.

#### Calculation of solvation energies from crystal morphology

At equilibrium, the morphology of a crystal is such that the surface free energy

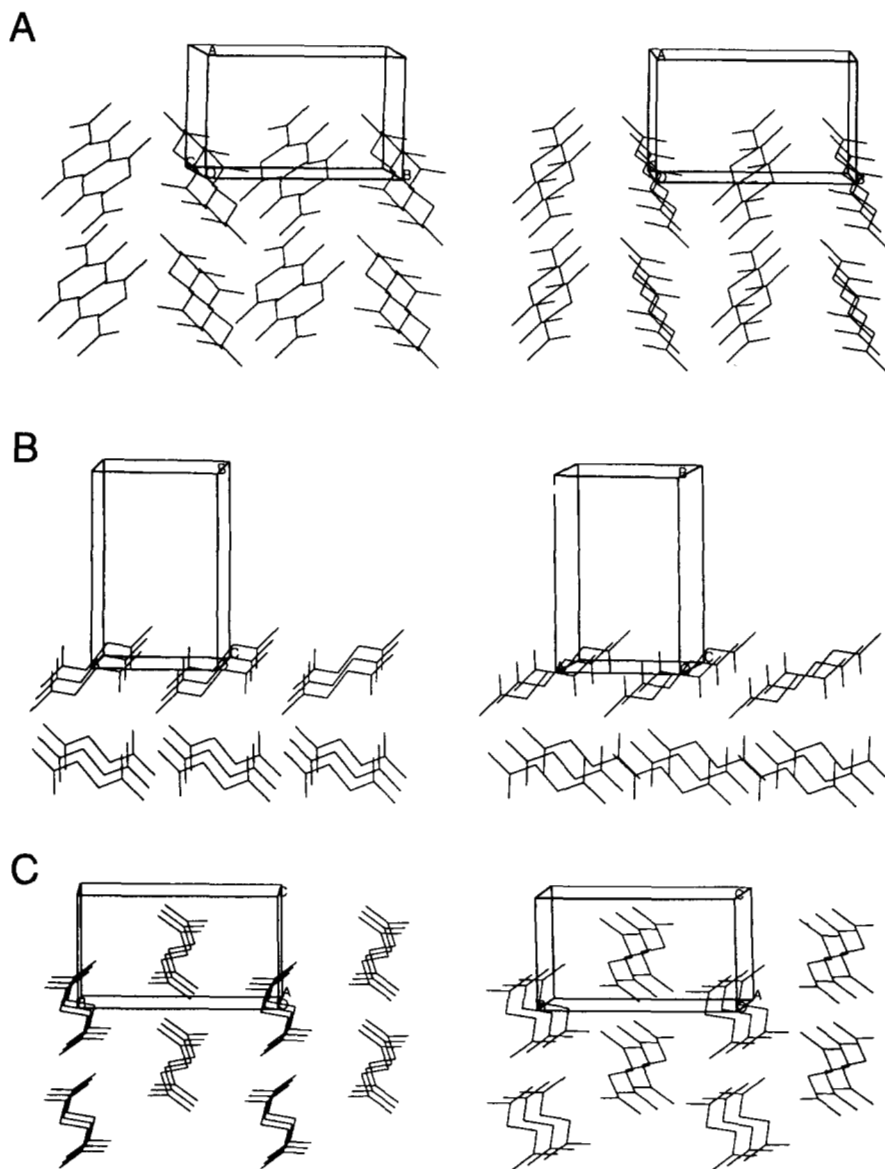
$$\Sigma A_i \gamma_i \quad (2)$$

is minimized, where  $A_i$  and  $\gamma_i$  are the surface area and surface free energy, respectively, of the  $i$ th crystal face (Gibbs, 1928). In general, crystal faces with low surface free energies (e.g., faces with exposed polar groups for crystals grown from aqueous solutions) will tend to have larger areas than faces with higher surface energies (e.g., faces with exposed nonpolar groups for crystals grown from aqueous solutions). The crystal morphology and the values of  $\gamma_i$  for each crystal face are related through a Wulff plot, equivalent to minimization of Equation 2 (Wulff, 1901; Dunning, 1963). In a Wulff plot, vectors of length  $\gamma_i$  are drawn normal to the  $i$ th crystal face. The planes perpendicular to these vectors enclose a polyhedron that exhibits the equilibrium crystal morphology. Given the  $\gamma_i$ , the relative values of  $A_i$  can be determined from a Wulff plot, and vice versa. Mathematically, the Wulff construction is analogous to the Voronoi polyhedra used to measure the volumes of atoms in proteins (Richards, 1974). The  $\gamma_i$  for each crystal face are related by the Dupré equation (Israelachvili, 1992) to the energies of solute–solute interactions at the specified crystal face ( $\gamma_S$ ; i.e., the free energy change required to create a unit area of crystal surface), the surface tension of the solution ( $\gamma_L$ ), and the work of adhesion between the solute and solution at the crystal face ( $W_{SL}$ ):

$$\gamma_i = \gamma_S + \gamma_L - W_{SL}. \quad (3)$$

$W_{SL}$  is the desired interaction energy between the solute and solvent at the crystal interface; an approach for deriving this term from small molecule crystal morphology is described below.

Succinic acid crystals are particularly suited for this type of analysis because: succinic acid contains both polar  $\text{CO}_2\text{H}$  and apolar  $\text{CH}_2$  groups that are representative of the atom types found in amino acid residues; the concentration of crystalline succinic acid is 13.3 M, which is comparable to concentrations of amino acid residues in proteins; the atomic structure of succinic acid crystals grown from aqueous solution has been determined (Leviel et al., 1981); the molecular and crystal symmetry is such that the surface structure of the different crystal faces is uniquely specified; various crystal faces differ in the relative exposure of the  $\text{CO}_2\text{H}$  and  $\text{CH}_2$  groups, as well as in the relative smoothness of the molecular packing along each face (Fig. 1) so that the influence of these factors on surface energetics may be evaluated; and the crystal morphology of succinic acid is solvent-dependent (Davey et al.,



**Fig. 1.** Stereo views of molecular packing at the (A) {100}, (B) {010}, and (C) {001} crystal faces of succinic acid. These packing arrangements illustrate the diversity in polarity and smoothness exhibited by different crystal faces. The {100} and {010} faces are observed in crystals grown from aqueous solution and are relatively smooth, whereas the {001} faces are not observed and are much rougher. The {100} faces consist primarily of exposed carboxyl groups and are more polar than the {010} faces that also expose the methylene carbons of succinic acid.

1982; Berkovitch-Yellin, 1985), demonstrating the importance of solute-solvent interactions in determining crystal morphology. Although the atomic positions in succinic acid molecules located at a crystal surface will necessarily differ from the bulk parameters, atomic force-microscopy studies of the surface structure of organic crystals (such as leucine) indicate that these deviations should be small (Gould et al., 1988). Accordingly, surface molecules are assumed in this work to maintain the bulk structure.

Crystals of succinic acid were grown by slow evaporation of saturated aqueous solutions. The relative areas of each crystal face were established by optically measuring the perpendicular distance of each face from a common point (as in determining the external geometry of a crystal for an absorption correction calculation [Ward & Caughlan, 1971]), followed by the Wulff plot construction described above. As reported by others, the {100},

{010}, {111}, {011}, and occasionally {110} forms dominate the morphology of succinic acid crystals grown from aqueous solutions (Mullin & Whiting, 1980; Leviel et al., 1981; Berkovitch-Yellin, 1985). The relative areas of these faces as measured by Mullin and Whiting (1980), Berkovitch-Yellin (1985), and ourselves are tabulated in Table 1. These represent the observed values of  $A_i$  that will be used to characterize the solvation energies of the  $\text{CH}_2$  and  $\text{CO}_2\text{H}$  groups through Equation 3. The general agreement in crystal morphology reported by different groups under differing conditions of crystal growth suggests (but does not prove) that the observed crystal morphologies approximate the equilibrium morphology, a necessary condition for using Equations 2 and 3 to relate the crystal morphology to surface energetics.

The energetic terms in Equation 3 were estimated or parameterized as follows. For molecular crystals, values of

**Table 1.** Morphology of succinic acid crystals<sup>a</sup>

Form	Relative crystal face area					$E_{att}$ (kcal/ mol)	$A_{cr}$ (Å <sup>2</sup> )	$\gamma_S$ (cal/ mol·Å <sup>2</sup> )
	MW	BY	GW	$\langle A_i \rangle$	$\sigma_i$			
{100}	0.69	0.49	0.21	0.46	0.24	13.90	22.6	308
{010}	0.10	0.04	0.09	0.08	0.03	12.34	28.1	220
{111}	0.06	0.20	0.67	0.31	0.32	15.03	36.6	205
{011}	0.15	0.20	0.03	0.13	0.09	17.23	28.2	305
{110}	0.0	0.07	0.0	0.02	0.04	17.52	26.6	329
{101}	0.0	0.0	0.0	0.0	0.01	19.64	33.8	291
{-111}	0.0	0.0	0.0	0.0	0.01	20.90	35.7	293
{001}	0.0	0.0	0.0	0.0	0.01	22.07	24.5	450

<sup>a</sup> The relative areas of the crystal faces labeled MW, BY, and GW were reported by Mullin and Whiting (1980), Berkovitch-Yellin (1985), and this paper, respectively.  $\langle A_i \rangle$  and  $\sigma_i$  designate the average and standard deviation, respectively, of the MW, BY, and GW areas. Each unobserved face was assigned  $\sigma_i = 0.01$ . The attachment energies ( $E_{att}$ ) were reported in Berkovitch-Yellin (1985). For comparison, the enthalpy of sublimation for succinic acid crystals is 28.1 kcal/mol (Davies & Thomas, 1960). The projected areas of the succinic acid unit cell in the indicated directions,  $A_{cr}$ , were calculated from the cell dimensions of succinic acid crystals grown at room temperature (space group  $P2_1/c$ ,  $a = 5.52$  Å,  $b = 8.86$  Å,  $c = 5.10$  Å,  $\beta = 91.59^\circ$  [Leviel et al., 1981]). Values of  $\gamma_S$  were calculated from Equation 4.

$\gamma_S$  may be obtained from  $E_{att}$ , the energy released per molecule when adding a layer of molecules onto a specified crystal face, from the relationship (Hartman & Bennema, 1980):

$$\gamma_S \sim E_{att}/(2A_{cr}), \quad (4)$$

where  $A_{cr}$  is the projected area per molecule for a given crystal face. Values of  $E_{att}$  for different crystal faces of succinic acid have been evaluated computationally using a potential energy function parameterized directly from crystals of carboxylic acids (Berkovitch-Yellin, 1985) and are listed in Table 1. With the assumption of group-additivity (commonly employed in analysis of transfer data, as exemplified by Equation 1),  $W_{SL}$  may be related to the polarity of the two types of groups found on crystal faces of succinic acid:

$$W_{SL} = \Delta\sigma_{CH_2}(A_{CH_2}/A_{cr}) + \Delta\sigma_{CO_2H}(A_{CO_2H}/A_{cr}), \quad (5)$$

where  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  are the surface free energies corresponding to exposed  $CH_2$  and  $CO_2H$  groups on the crystal surface. The exposed areas of these groups ( $A_{CH_2}$  and  $A_{CO_2H}$ ) on different crystal faces may be evaluated using either the ASA or MSA (Table 2). The ASA were calculated using Richmond's (1984) implementation of the Lee and Richards (1971) algorithm, whereas the MSA were obtained from Connolly's (1983) MS program. Probe, carbon atom, and oxygen atom radii were assigned values of 1.4, 1.8, and 1.4 Å, respectively, for all surface area calculations. Hydrogen atoms were omitted from the

**Table 2.** Exposed surface area calculations for the  $CH_2$  and  $CO_2H$  groups along different faces of succinic acid crystals

Form	MSA			ASA	
	$A_{cr}$ (Å <sup>2</sup> )	$A_{CH_2}$ (Å <sup>2</sup> )	$A_{CO_2H}$ (Å <sup>2</sup> )	$A_{CH_2}$ (Å <sup>2</sup> )	$A_{CO_2H}$ (Å <sup>2</sup> )
{100}	22.6	0.0	25.3	0.0	28.4
{010}	28.1	10.9	19.5	6.5	26.9
{111}	36.6	13.1	26.5	10.3	31.4
{011}	28.2	10.8	25.8	2.8	36.7
{110}	26.6	5.8	30.0	3.1	39.9
{101}	33.8	13.3	26.3	12.0	30.0
{-111}	35.7	11.0	36.0	2.8	58.7
{001}	24.5	10.8	25.8	3.5	35.9
Isolated molecule		32.6	72.8	56.9	195.1

coordinate list for consistency with a typical macromolecular surface area calculation. The surface tension for the aqueous solution surrounding the crystal,  $\gamma_L$ , was given the value 104 cal/Å<sup>2</sup> (the surface tension of pure water), corrected by the factor that the actual surface area of the crystal structure differs from the projected area:

$$\gamma_L = 104[(A_{CH_2} + A_{CO_2H})/A_{cr}] \text{ cal/Å}^2. \quad (6)$$

Values of  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  were determined by numerically minimizing the quantity  $\chi^2$ :

$$\chi^2 = \sum_{i=1}^{\text{no. faces}} \left\{ \frac{[A_i(\text{obs}) - A_i(\text{calc})]}{\sigma_i} \right\}^2, \quad (7)$$

where  $A_i(\text{obs})$  and  $A_i(\text{calc})$  are the observed and calculated fractional areas of the  $i$ th crystal face, and the  $\sigma_i$  are the experimental standard deviations of the  $A_i(\text{obs})$  (Table 1). The  $A_i(\text{calc})$  are obtained from a Wulff plot, using values of  $\gamma_i$  calculated as a function of  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  from Equation 3. Using MSAs, the optimal values of  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  established by this procedure are +65 cal/Å<sup>2</sup> and +370 cal/Å<sup>2</sup>, respectively, with  $\chi^2 = 15.4$  when summed over all the crystal faces in Table 1. An estimate of the error in these parameters is provided by the variation observed in  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  when the three different sets of  $A_i$  measurements compiled in Table 1 are used to independently minimize  $\chi^2$ . In this fashion, the ranges in  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  values are observed to be 40–80 cal/Å<sup>2</sup> and 370–375 cal/Å<sup>2</sup>, respectively. The value of  $\Delta\sigma_{CO_2H}$  is better defined than that of  $\Delta\sigma_{CH_2}$  because the carboxyl group dominates the surface area of the different succinic acid crystal faces, and the large value of  $\Delta\sigma_{CO_2H}$  quantitatively dominates the value of  $W_{SL}$  calculated from Equation 5. In contrast to the use of MSAs, it was not possible to estimate values for  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$  using ASAs. With ASAs, a minimum

value of  $\chi^2 = 152$  was obtained when both  $\Delta\sigma_{\text{CH}_2}$  and  $\Delta\sigma_{\text{CO}_2\text{H}}$  were equal to zero. This behavior may reflect the relatively small values of  $A_{\text{CH}_2}$  calculated for all crystal faces using ASAs and the large values of  $A_{\text{CO}_2\text{H}}$  calculated with ASAs for unobserved crystal faces (such as the  $\{-111\}$  form), with the consequence that these faces were predicted from Equation 3 to have low surface energies. Due to the inability to estimate values for  $\Delta\sigma_{\text{CH}_2}$  and  $\Delta\sigma_{\text{CO}_2\text{H}}$  using ASAs, the following discussion will focus on values obtained using MSAs.

### Discussion

Values for  $\Delta\sigma_{\text{CH}_2}$  and  $\Delta\sigma_{\text{CO}_2\text{H}}$  derived above using MSAs may be checked for consistency with independent measurements of solvation energies derived from both macroscopic and microscopic systems.

#### Contact angle studies

The interaction energy,  $W_{\text{SL}}$ , between either  $\text{CH}_2$  or  $\text{CO}_2\text{H}$  groups and water may be evaluated directly in macroscopic systems by measurement of the contact angle,  $\theta$ , formed by a water drop sitting on the appropriate surface (Israelachvili, 1992). The relationship between these two quantities is provided by the Young-Dupré equation (Israelachvili, 1992):

$$W_{\text{SL}} = \gamma_{\text{L}}(1 + \cos \theta). \quad (8)$$

For pure methylene surfaces,  $W_{\text{SL}} = \Delta\sigma_{\text{CH}_2}$  (Equation 4), so that  $\theta$  is predicted to be  $112^\circ$  from Equation 8, using the value  $\Delta\sigma_{\text{CH}_2}$  of  $65 \text{ cal}/\text{\AA}^2$  derived from the crystal morphology analysis. Significantly,  $W_{\text{SL}} = \Delta\sigma_{\text{CH}_2} = 65 \text{ cal}/\text{\AA}^2$  is in good agreement with the macroscopic hydrocarbon-water interfacial free energy of  $72 \text{ cal}/\text{\AA}^2$  (Tanford, 1979). For pure carboxyl surfaces,  $W_{\text{SL}} = \Delta\sigma_{\text{CO}_2\text{H}}$ , and Equation 8 predicts complete wetting of the surface by water, i.e.,  $\theta = 0^\circ$ . Relevant experimental values of  $\theta$  have been reported to be  $\sim 105^\circ$  for pure methylene surfaces (Adam, 1964),  $112^\circ$  for paraffin (Israelachvili, 1992),  $112^\circ$  for methyl-exposed monolayers (Holmes-Farley et al., 1988; Bain & Whitesides, 1988, 1989), and  $< 10^\circ$  for carboxyl-exposed monolayers (Holmes-Farley et al., 1988; Bain & Whitesides, 1989), which are consistent with the predicted values. Contact angle studies provide a well-established method for determining solute-solvent interaction energies of macroscopic surfaces, and the agreement between these results and those derived from analysis of crystal morphology (another macroscopic system) indicates that the latter approach is also useful for measuring solvation energies.

#### Transfer free energies

A connection may also be established between solvation energies derived from crystal morphology and those ob-

tained from transfer free energy measurements. The free energy change associated with transferring an existing surface from a vacuum to water,  $\Delta G_{\text{VL}}$ , is given by (Israelachvili, 1992):

$$\Delta G_{\text{VL}} = \gamma_{\text{L}} - W_{\text{SL}}. \quad (9)$$

For pure methylene and carboxyl surfaces transferred to water ( $\gamma_{\text{L}} \sim 104 \text{ cal}/\text{\AA}^2$ ),  $\Delta G_{\text{VL}}$  values of approx.  $+40$  and  $-260 \text{ cal}/\text{\AA}^2$ , respectively, are expected on the basis of the  $\Delta\sigma_{\text{CH}_2}$  and  $\Delta\sigma_{\text{CO}_2\text{H}}$  values derived from the crystal morphology analysis. With the MSAs of exposed methylene and carboxyl groups in various carboxylic acids,  $\Delta G_{\text{VL}}$  may be calculated using these values ( $+40$  and  $-260 \text{ cal}/\text{\AA}^2$ , respectively) in Equation 1. Experimental values of  $\Delta G_{\text{VL}}$  may be obtained from the distribution coefficients of compounds between the vapor phase and aqueous solution, using Wolfenden's approach (Wolfenden, 1978; Wolfenden et al., 1981). A comparison between these observed and calculated values of  $\Delta G_{\text{VL}}$  is provided in Table 3 for several carboxylic acids. Overall, the quantitative agreement between the observed and calculated values seems satisfactory.

Although it was not possible to derive ASA-based  $\Delta\sigma$  values from the morphology of succinic acid crystals, corresponding parameters can be estimated for ASA-based values of  $\Delta G_{\text{VL}}$ . To a first approximation, MSAs and ASAs are proportional (Chothia, 1984); for the isolated succinic acid molecule, the overall ASA is greater than the MSA by a factor of 2.4 (Table 2). Hence,  $\Delta G_{\text{VL}}$  based on ASAs would be  $\sim 2.4$  times smaller than those derived using MSAs. Applying this factor to the MSA-based values of  $\Delta G_{\text{VL}}$  for methylene and carboxyl surfaces ( $+40$  and  $-260 \text{ cal}/\text{\AA}^2$ , respectively) results in values of  $+17$  and  $-108 \text{ cal}/\text{\AA}^2$  for the ASA-based values of  $\Delta G_{\text{VL}}$  for methylene and carboxyl surfaces, respectively. These values are in good agreement to the atomic solvation parameters of  $+12$  and  $-116 \text{ cal}/\text{\AA}^2$  reported by Wesson and Eisenberg (1992) for the transfer from vacuum to water of carbon and uncharged oxygen atoms, respectively, using ASAs and correcting the experimental free energies of transfer as described by Sharp et al. (1991b).

The general agreement between solvation energies derived from vapor-water transfer experiments in dilute solutions and those obtained from analysis of macroscopic systems (contact angle measurements and crystal morphology) implies that transfer free energies provide a reasonable estimate of solvation interactions of macromolecules. Conversely, the ability to directly determine these interaction energies from contact angle measurements on an impressive array of functionalized monolayers (Bain & Whitesides, 1989) suggests that contact angle studies offer an attractive approach for quantitating solvation energies of macromolecules. Direct measurements of these solute-water interaction energies are especially important because they yield the total energy of interaction between

**Table 3.** Comparison of observed and calculated values of the free energy of transfer from vacuum to water,  $\Delta G_{VL}$ , for different carboxylic acid containing compounds<sup>a</sup>

Compound	$\Delta G_{VL}$ , obs (kcal/mol)	MSA			ASA		
		$A_{CH_2}$ ( $\text{\AA}^2$ )	$A_{CO_2H}$ ( $\text{\AA}^2$ )	$\Delta G_{VL}$ , calc (kcal/mol)	$A_{CH_2}$ ( $\text{\AA}^2$ )	$A_{CO_2H}$ ( $\text{\AA}^2$ )	$\Delta G_{VL}$ , calc (kcal/mol)
Acetic acid	-6.8	29.4	37.5	-8.6	75.5	104.2	-
Propionic acid	-6.5	44.6	36.6	-7.7	107.9	97.6	-
Succinic acid	-14.6	32.6	72.8	-17.6	56.9	195.1	-
Adipic acid	-14.3	62.2	70.9	-15.9	111.2	195.2	-
Suberic acid	-14.3	96.9	71.2	-14.6	169.1	195.3	-
Sebacic acid	-14.5	129.0	70.2	-13.1	223.4	185.6	-

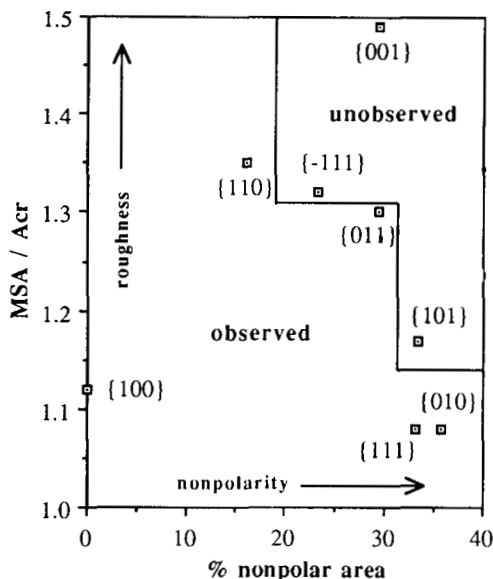
<sup>a</sup> Experimental values were calculated from the expression  $\Delta G_{VL} = -RT \ln(X_{\text{vap}}/X_{\text{H}_2\text{O}})$ , where  $X_{\text{vap}}/X_{\text{H}_2\text{O}}$  represents the distribution coefficients for the various acids (mol/L in the vapor phase divided by mol/L in water [Wolfenden, 1978]). No corrections for ionization effects were included. Values for acetic and propionic acids were taken from Wolfenden (1978), while vapor and aqueous phase concentrations for the dicarboxylic acids were reported in Saracco and Spaccamela Marchetti (1958), Davies and Thomas (1960), and Budavari (1989). MSAs and ASAs were calculated as described in the text. Equations 1 and 9 were used to calculate  $\Delta G_{VL}$ , using MSA-derived values for  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$ . Due to the inability to estimate values for ASA-based  $\Delta\sigma_{CH_2}$  and  $\Delta\sigma_{CO_2H}$ , corresponding calculated values for  $\Delta G_{VL}$  could not be obtained.

the solute and water, relative to a vacuum reference state. Consequently, these energies are well suited for incorporation into molecular mechanics programs that attempt to estimate the total energy of a macromolecule (Wesson & Eisenberg, 1992).

Provided the MSAs of the solute molecules are used, there is rather good agreement between solvation energies obtained from contact angle measurements, transfer free energies, and analysis of crystal morphology. Although ASAs are traditionally used for defining and characterizing macromolecular surfaces, there are several reasons to prefer the use of MSAs, especially in the analysis of complementary surfaces: (1) Due to displacement of the solvent-accessible surface from the molecular surface, different values for the ASA will be obtained depending on which of the two complementary surfaces is used in the calculation. An extreme (but common) case involves the ASA of a cavity in a protein of sufficient size to include a single water molecule (radius = 1.4  $\text{\AA}$ ). With a probe radius of 1.4  $\text{\AA}$ , the ASA of the cavity is 0  $\text{\AA}^2$  (calculated from the protein atoms), whereas the ASA of the water molecule that fits in the cavity is  $4\pi(1.4 + 1.4)^2 = 98.5 \text{\AA}^2$ . In addition, the two surfaces will be displaced by 2.8  $\text{\AA}$ . In contrast, the molecular surface defined by both the protein atoms surrounding the cavity and the enclosed water molecule will be more nearly equivalent, and each will have MSAs close to  $4\pi(1.4)^2 = 24 \text{\AA}^2$ . The relative independence of the molecular surface generated by complementary groups of atoms, compared to the solvent-accessible surface, is an advantage in problems involving molecular docking. (2) The MSA is better suited to analysis of surface roughness based on the variation in area with probe radii (Lewis & Rees, 1985). Due to displacement of the accessible surface from the van der Waals surface, there is a trivial contribution to the variation in ASA with probe radii that is independent of surface roughness.

As an example, the MSA of a smooth sphere of radius  $R$  measured with a probe of radius  $r$  is always  $4\pi R^2$ , whereas the ASA has the value  $4\pi(R+r)^2$ . The MSA has the advantage of providing a well-defined value for well-defined surfaces and reflects less contribution from the probe radius. (3) Because the MSAs are smaller than the ASAs, values of  $\Delta\sigma$  calculated from transfer free energies using Equation 1 will be larger when MSAs are used than when ASAs are used, which will help diminish the discrepancy between microscopic and macroscopic values of hydrocarbon-water interfacial energies noted in Tanford (1979) and Sharp et al. (1991a). Together with the present results on the energetic analysis of crystal surfaces, there appear to be significant advantages to the standard use of MSAs in the calculation of macromolecular surfaces areas.

Surface roughness can influence solvation energies observed at the surface-water interface through two mechanisms: (1) Surface roughness will influence the strength of intermolecular interactions between solute molecules (this is the basis of the periodic bond chain model of crystal morphology [Hartman, 1963]), and (2) surface roughness will influence the solvent-solute interaction by altering the surface area exposed to the solvent. The surface roughness of crystal faces of succinic acid may be estimated from the ratio of the MSA to the projected crystallographic area per molecule ( $A_{cr}$ ) exposed on different crystal faces. These quantities are plotted in Figure 2 as a function of the fraction of exposed surface contributed by methylene groups (which provides a measure of the relative apolarity of the surface). The dominant forms of succinic acid crystals grown from aqueous solution (Table 1), which have the lowest surface energy, are the {100} and {111} forms, which are both smooth. Additionally, the {100} form is also quite polar. Unobserved forms (having high surface energies) are characterized by combined apolarity and roughness of the surface,



**Fig. 2.** Relationship between the relative apolarity and surface smoothness for different crystal faces of succinic acid. The relative apolarity is defined by the percentage of the MSA of a crystal face contributed by  $\text{CH}_2$  groups. Surface smoothness is measured by the ratio of  $\text{MSA}/A_{\text{cr}}$  for different crystal faces. The superimposed lines have no theoretical significance; they only serve to separate observed and unobserved faces in succinic acid crystals grown from aqueous solution.

whereas less prominent but observed forms, such as {011} and {110}, tend to have surfaces that are either rough and polar (the {011} and {110} forms) or smooth and apolar (the {010} form). In general, it appears that increasing surface roughness is associated with increasing surface energy for a particular crystal face. This behavior is consistent with the observed surface roughness of the subunit interface regions of proteins (which are surface regions of a monomer that are buried from solvent exposure in the oligomer), relative to other regions of the protein surface (Lewis & Rees, 1985). The burial of the interface region away from solvent exposure is equivalent to the statement that these rough, relatively apolar surfaces have a high surface energy for interaction with water. This indicates that modification of surface polarity and smoothness of protein surfaces provides a mechanism for altering surface energies, thereby modulating the surface and solubility properties of these molecules.

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