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FOR THE RECORD

Polarity of disulfide bonds

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Protein stability is defined as the free energy of denatured state minus that of the native state, ΔG_d. Flory (1956) proposed that disulfide bonds increase ΔG_d by decreasing chain entropy in the denatured state. Alternatively, Doig and Williams (1991) suggest that the increase in ΔG_d of proteins by disulfide bonds is primarily enthalpic. Whatever the source of the stabilization, proteins with naturally occurring disulfide bonds are more stable than their noncross-linked equivalents (Pace et al., 1988). Introduction of novel disulfides, however, does not always stabilize proteins (Betz & Pielak [1992] and references therein).

Disulfide bonds are often buried within proteins (Thorton, 1981), and there is a correlation between the extent that residues are buried and their polarity (Rose et al., 1985; Miller et al., 1987). Therefore, we wondered whether a difference in polarity between two cysteines and a cystine could account, in part, for the stabilizing effect of disulfide bonds. To this end, the distribution coefficient between cyclohexane and H_2O, $K_{chx \rightarrow H_2O}$, of a cystine analog, methyl disulfide (CH_3-S-S-CH_3), was measured. The $K_{chx \rightarrow H_2O}$ value for the cysteine analog, methanethiol (CH_3-SH), was reported by Radzicka and Wolfenden (1988). As a control, log $K_{chx \rightarrow H_2O}$ for ethyl methyl sulfide (CH_3-CH_2-S-CH_3) was determined so that it could be compared to the value, −1.73, reported by Radzicka and Wolfenden.

There is excellent agreement between the data for ethyl methyl sulfide (Table 1) and that previously reported. This gives us confidence in comparing our data for CH_3-S-S-CH_3 to that for CH_3-SH (Table 1). Comparison of the free energy of transfer between cyclohexane and H_2O ($\Delta G_{tr} = -RT \ln K_{chx \rightarrow H_2O}$) for these analogs suggests that at room temperature the burial of a disulfide is favored over the burial of two cysteines by 0.5 kcal mol^{-1}. This difference can account for nearly 20% of the predicted increase in stability supplied by the average disulfide bond (Pace et al., 1988).

Table 1. *The polarity of sulfur-containing side-chain analogues in cyclohexane at room temperature*

	Solute		
	CH_3-CH_2-S-CH_3[a]	CH_3-S-S-CH_3[a]	CH_3-SH[b]
log $K_{chx \rightarrow H_2O}$	−1.72 ± 0.07	−2.21 ± 0.04	−0.93
ΔG_{tr} (kcal mol^{-1})	2.33 ± 0.10	2.99 ± 0.06	1.28

[a] Distribution coefficients were determined at 296 K using solvents that had been equilibrated against each other. A 1.00-mL aliquot of a 1.00 M solution of solute in cyclohexane was equilibrated against 99.0 mL of H_2O. After equilibration (rapid stirring for 4 h), phases were allowed to separate and the cyclohexane phase was removed. To recover the solute from the H_2O phase, a 20.0-mL back extraction was performed. The back extraction recovered greater than 95% of the solute. Concentrations were determined by proton magnetic resonance spectroscopy using a Bruker AMX500 with a 30-s delay between pulses. Samples contained 10% ^{2}H-cyclohexane, 90% ^{1}H-cyclohexane, and 100 μmol of pyrazine. Distribution coefficients were calculated by dividing the concentration of solute in the H_2O phase by the concentration of solute in the cyclohexane phase. Each value is the average of three determinations and is reported with its standard deviation.

[b] Radzicka and Wolfenden (1988).

In summary, these data show that a disulfide bond is less polar than two cysteines. This observation should be considered when evaluating the effect of disulfides on protein stability.

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