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On the Role of Methionine Residues in the Sequence-Independent Recognition of Nonpolar Protein Surfaces†

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Exquisite discrimination among closely related structures is a hallmark of biochemical systems, but it has recently become clear that some crucial intermolecular recognition processes are guided only by general structural features. Signal peptides are interesting in this regard, because their information content is not dependent upon a precise amino acid sequence (Gierasch, 1989; Randall & Hardy, 1989). The proper targeting functions of signal peptides can be retained after dramatic changes in sequence, so long as the overall nonpolar nature of the constituent residues is maintained (Kaiser et al., 1987; Kendall et al., 1990; Yamamoto et al., 1989). If the message carried by signal peptide fragments is contained in the net physical character of the peptide rather than in a precise sequence, how are the many different but functionally equivalent signal sequences perceived as synonyms, and distinguished from other sequences, by the relevant biological receptor or receptors?

Bernstein et al. (1989) recently suggested an intriguing answer to this question. In the process of examining the targeting of proteins to the endoplasmic reticulum (ER) in mammalian cells, these workers determined the sequence of cDNA for the MW 54 000 subunit of the signal-recognition particle (SRP54). SRP binds to the signal sequence at the N-terminus of an ER-bound nascent peptide chain as that chain emerges from the ribosome (Rapoport, 1990; Rothman, 1989). This binding event temporarily blocks further translation, until SRP interacts with the SRP receptor protein that is embedded in the ER membrane. Once the nascent protein chain and the attached ribosome and mRNA have been delivered to the ER membrane, SRP dissociates and translation starts up again, now with concomitant translocation of the protein chain into the lumen of the ER.

The sequence deduced by Bernstein et al. (1989) for SRP54 implicates two structural domains, one of which is unusually rich in methionine residues. Further analysis of this so-called M-domain, through the use of secondary structure prediction algorithms, led these workers to propose that the M-domain contains three amphiphilic α-helices, each of which bears several methionine residues on its nonpolar face. Previous data had indicated that SRP54 is the SRP subunit that interacts directly with the N-terminus emerging from the ribosome, and Bernstein et al. (1989) proposed that the clusters of methionine residues play a key role in a recognition process that must be able to accept a wide variety of nonpolar peptide sequences. These workers noted that "a unique structural feature of the Met side chains is their flexibility", in contrast, the side chains of leucine and isoleucine, residues of comparably low polarity, "are branched and hence comparatively rigid". On the basis of these general features, it was proposed that the methionine side chains on one face of each amphiphatic helix provide a malleable nonpolar surface that can adapt itself to peptide binding partners of varying dimensions.

O'Neil et al. (1989) have suggested a similar mechanism for a very different set of protein–protein interactions. Calmodulin associates with a wide variety of protein partners; although the sequences of calmodulin's many binding partners are divergent, most contain an amphiphilic α-helix. O'Neil et al. (1989) pointed out that calmodulin's binding site contains eight exposed methionine residues and that this group of methionine side chains provides a plastic surface that can conform to structurally diverse nonpolar surfaces on binding partners. Like the SRP54 investigators, O'Neil and DeGrado (1990) rationalized this role for methionine by commenting that "unlike other hydrophobic residues, the side chain of Met is unbranched providing considerable conformational flexibility".

My purpose here is to amplify and modify the hypothesis of Bernstein et al. (1989) and of O'Neil et al. (1989) by pointing out that two properties of the sulfur-containing side chain in addition to its lack of branching are probably crucial to the special role of methionine residues in the sequence-in-

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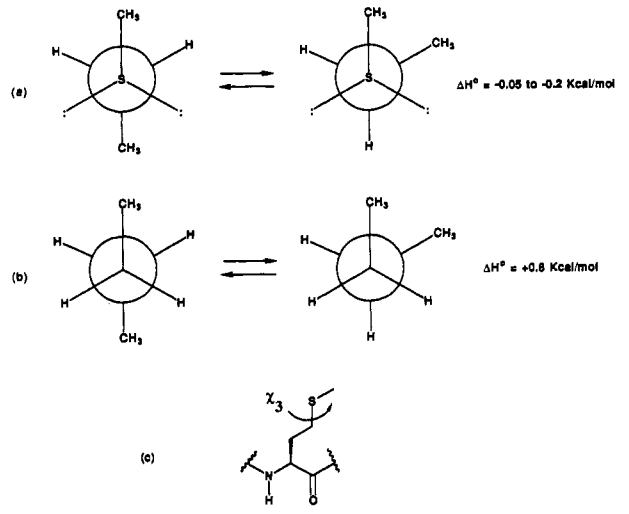

FIGURE 1: (a) Anti and gauche conformations of $CH_3SCH_2CH_3$, as viewed in a Newman projection down the central S–C bond. (b) Anti and gauche conformations of $CH_3CH_2CH_2CH_3$, as viewed in a Newman projection down the central C–C bond. (c) A methionine residue, with the χ_3 torsion angle of the side chain indicated.

dependent recognition of nonpolar protein surfaces. One of these properties is essential in promoting the structural "plasticity" identified by Bernstein et al. (1989) and O'Neil et al. (1989); the other property has a very different impact on the protein–protein association process.

Origins of Side-Chain Flexibility. The side chain of methionine is indeed more flexible than the side chains of leucine or isoleucine, but this flexibility does not result exclusively from differences in branching. Methionine's structural plasticity arises largely from the unusual properties of the CH_2S–C-H_2CH_2 torsional unit (the χ_3 torsion angle of the methionine side chain involves a CS–CC unit). The CS–CC unit shows relatively little enthalpic preference between gauche and anti conformations, with gauche slightly favored. This preference amounts to 0.05–0.20 kcal/mol for 2-thiobutane (CH_3SC-H_2CH_3) in the gas phase (Durig et al., 1979; Oyanagi & Kuchitsu, 1978; Sakakibara et al., 1977). In contrast, for butane ($CH_3CH_2CH_2CH_3$) the anti conformation is enthalpically favored by 0.8 kcal/mol in the gas phase (Allinger et al., 1989). (These results are summarized in Figure 1.) Thus, butane is a relatively rigid extended rod, while 2-thiobutane is a floppy molecule, just barely preferring the more compact gauche form. Examination of 2-thiobutane and other small thioethers in the liquid and solid states reveals the presence of both gauche and anti CS–CC torsion angles (Hayashi et al., 1966; Nogami et al., 1975; Ogawa et al., 1977; Ohta et al., 1977). A preference for gauche CS–CC torsion angles has been detected crystallographically in macrocyclic (Wolf et al., 1987) and acyclic (Desper et al., 1990) polythioethers.

It is not clear why CS–CC torsional units modestly prefer gauche over anti conformations. The difference in C–C vs C–S bond lengths (1.5 vs 1.8 Å) leads to a decrease in the steric repulsion between the terminal methyl groups of the gauche rotamer of 2-thiobutane, relative to the steric repulsion between the terminal methyl groups of the gauche rotamer of butane itself. This diminution of steric repulsion is certainly an important factor, but there must be an additional and as yet unidentified feature of the CS–CC unit that promotes the gauche conformation.

A statistical survey of crystalline proteins has revealed that the distribution of methionine χ_3 torsion angles is virtually flat over the entire range of possible values, in contrast to the pronounced periodicities observed for all other amino acid side-chain torsion angles (Janin et al., 1978). If Nature had chosen norleucine (*n*-butyl side chain) as one of its protein building blocks, a substantial χ_3 preference for 180° would have been expected for this residue. Norleucine χ_3 torsion angles at ±60° would have been less common, and χ_3 torsion angles approaching the eclipsed values of ±120° would have been very unlikely. [For butane itself, the conformation with methyls eclipsing hydrogens is estimated to be 3.3 kcal/mol higher in enthalpy than the anti conformational ground state (Allinger et al., 1989).]

Thus, the substantial flexibility of the methionine side chain probably depends more on the presence of the sulfur atom than on the lack of branching. Since there is little enthalpic discrimination among the possible χ_3 torsion angles for methionine, protein surfaces containing this residue have considerable freedom to mold themselves to accommodate nonpolar binding partners of varying structures. In contrast, a protein surface containing the unbranched side chains of norleucine residues would be much less adaptable, since it would cost 0.8 kcal/mol to change χ_3 from anti to gauche and it would cost considerably more to distort this torsion angle toward eclipsed values.

Dispersion Forces in Protein–Protein Association. Methionine's special role in protein recognition probably also stems from the nature of the interactions that lead to affinity between nonpolar surfaces in aqueous solution. There has been considerable debate on the relative importance of the "hydrophobic effect", dispersion forces, and other noncovalent interactions in protein folding (Baldwin, 1986; Dill, 1990; Livingstone et al., 1991; Muller, 1990; Murphy et al., 1990; Privalov & Gill, 1988, 1989; Spolar et al., 1989). Since the forces that induce a single protein chain to collapse upon itself are likely to be similar to the forces responsible for one protein adhering to another, the points of contention in the folding debate are relevant to our subject. In the following discussion, it is important to bear in mind that the large polarizability of the side-chain sulfur atom makes methionine residues especially "sticky" when dispersion forces are at work.

When nonpolar moieties associate with one another in aqueous solution, does the aggregation result because they are mutually excluded from water or because the nonpolar moieties experience a selective attraction for one another? Water interacts more favorably with itself, via strong hydrogen bonding, than it does with nonpolar surfaces, so nonpolar moieties tend to be squeezed out of bulk aqueous solution—this is the mechanism historically associated with the term "hydrophobic effect" (Kauzmann, 1959; Tanford, 1980; Muller, 1990). The clustering of nonpolar groups in aqueous solution, however, may be more than a marriage of convenience. Nonpolar moieties are typically more polarizable than water. Since the dispersion attraction between two molecular surfaces is proportional to each surface's polarizability, the dispersion attraction between two nonpolar fragments should be greater than the dispersion attraction between a water molecule and a nonpolar fragment.

For transfer of simple hydrocarbons from a nonpolar environment to aqueous solution at room temperature, one generally observes an unfavorable $\Delta S°$, $\Delta H°$ close to zero, and a positive $\Delta C_p°$ (Jencks, 1969; Muller, 1990). The molecular picture commonly invoked to explain this thermodynamic profile is that the water molecules in contact with the nonpolar solutes have become more highly ordered than bulk water. The increased water order is entropically unfavorable, but the ordering allows the surface water molecules to maintain a high degree of hydrogen bonding with neighboring water molecules, thus minimizing the enthalpic penalty of hydrating a surface

that cannot form hydrogen bonds. Enthalpic neutrality at room temperature is often considered to be a hallmark of processes that involve the hydrophobic effect. Because the enthalpy of transfer for simple hydrocarbons from the pure liquid to dilute aqueous solution is small (at room temperature), it is commonly assumed that any loss of dispersion attraction among the hydrocarbon groups, which should be manifested as an unfavorable enthalpy, is not of primary importance in the transfer process. The validity of this reasoning is unclear, however, since favorable enthalpies of transfer are observed at higher temperatures. [Indeed, it has been argued that $\Delta S°$, $\Delta H°$, and $\Delta G°$ are not useful parameters for the identification of hydrophobically driven processes, and that the most reliable indicator is a large and negative $\Delta C_p°$ (Tanford, 1980; Spolar et al., 1989; Ha et al., 1989).] Furthermore, data from more sophisticated model systems for biopolymer interactions show that large enthalpy changes *are* manifested at room temperature for some complexation phenomena that involve hydration or dehydration of nonpolar surfaces. These results imply that the entropy-driven hydrophobic effect is not the only important factor in such processes.

Thermodynamic analysis of the complexation of small, nonpolar organic molecules by cyclodextrins in aqueous solution often reveals a large favorable enthalpy term for complex formation at room temperature (Eftink et al., 1989; Harrison & Eftink, 1982; Matsui et al., 1985). Similar observations have recently been reported for the complexation of aromatic guests by synthetic molecules ("cyclophanes") containing well-defined nonpolar binding pockets (Ferguson et al., 1988). In a few of the cyclodextrin cases, $\Delta C_p°$ has been measured for such binding events, and the values have been found to be relatively large and negative (Harrison & Eftink, 1982). A number of explanations for the substantial favorable enthalpy that characterizes these binding events have been offered, the most widely accepted of which involves London dispersion forces (Ferguson et al., 1988; Harrison & Eftink, 1982; Tabushi et al., 1978; Wojcik, 1984). The interior surface of the cyclodextrin or cyclophane cavity is lined largely by CH moieties, which have a substantially higher polarizability than do water molecules. Therefore, these nonpolar molecular cavities should display a greater dispersion-based affinity for hydrocarbons than for water molecules.

Various research groups have attempted to dissect the binding forces between cyclodextrins and small organic "guests" into hydrophobic and dispersion attraction components (Eftink et al., 1989; Matsui & Mochida, 1979). In general, these efforts have led to the conclusion that the contribution of dispersion forces between the cyclodextrin and its partner is largest when the guest fits snugly, but not too tightly, into the cavity. In such cases, there is maximal nonpolar surface contact without steric repulsion, and dispersion forces appear to be the dominant source of the intermolecular attraction.

The polarizability of sulfur in its lower oxidation states is substantially larger than the polarizabilities of typical hydrocarbon moieties (Fersht, 1985). Since the protein–protein interactions involving SRP54 and calmodulin appear to involve tight association of molecular surfaces, one might expect London dispersion forces between the binding partners to make a significant contribution to the net attraction. Incorporation of multiple methionine residues into a binding site should therefore be a good way to optimize that site's affinity for partners with extensive nonpolar surfaces.

Conclusion. The thioether sulfur atom in the methionine side chain is not simply an exotic methylene equivalent. Nature apparently takes advantage of the unique conformational properties of thioether fragments, and the unusually large polarizability of the sulfur atom itself, in generating binding sites that are tailored for strong interactions with nonpolar surfaces on binding partners and that can adapt themselves to partners of different shapes.

One is tempted to speculate about other ways in which the unique properties of the thioether sulfur atom might be employed to achieve specialized functional ends. For example, the nonpolar thioether moiety can be converted to a very polar group by conversion to the sulfoxide. Conversion of thioethers to sulfoxides can be carried out be a variety of oxidative enzymes, and it is imaginable that biological systems make use of this transformation to alter the polarity of protein surfaces. The ability to reduce sulfoxides enzymatically would endow a biological system with a very powerful way to modulate reversibly the hydrophobic/hydrophilic balance of the surfaces of methionine-containing proteins.

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Articles

Characterization of the R-State Insulin Hexamer and Its Derivatives. The Hexamer Is Stabilized by Heterotropic Ligand Binding Interactions†

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ABSTRACT: ^{1}H NMR and UV-visible electronic absorption studies have been performed to investigate the effects of anions and cyclic organic molecules on the interconversion of the T- and R-conformational states (Kaarsholm et al., 1989) of hexameric M(II)-substituted insulin in solution (M = Zn or Co). Two ligand binding processes that stabilize the R-state conformation of the M(II)-substituted insulin hexamer [M(II)-R_6] have been distinguished: (i) The binding of neutral organic molecules to the six, crystallographically identified, protein pockets in the Zn(II)-R_6 insulin hexamer (Derewenda et al. 1989) generate homotropic site-site interactions that stabilize the R-state. Cyclohexanol, phenol, 4-nitrophenol, and 4-hydroxymethylbenzoate are shown to bind at these sites. (ii) The coordination of singly charged anions that are able to gain access to the two HisB10 coordinated metal ions of the M(II)-R_6 hexamer stabilizes the R-state. Adducts of the M(II)-R_6 hexamer are formed, thereby, in which the solvent-accessible fourth coordination position of the M(II) ion is replaced by a competing anion. Binding to these two classes of sites introduces strong heterotropic interactions that stabilize the R-state. UV-visible spectral data and apparent affinity constants for the adducts formed by the Co(II)-R_6 hexamer with a wide range of anionic ligands are presented. The Co(II)-R_6 adducts have a strong preference for the formation of pseudotetrahedral Co(II) centers. The HCO_3^- and pyridine-2-thiolate ions form Co(II)-R_6 adducts that are proposed to possess pentacoordinate Co(II) geometries. The relevance of the Co(II)-R_6 complexes to carbonic anhydrase catalysis and zinc enzyme model systems is discussed.

Insulin is synthesized in the β-cells of the pancreas where it is stored as crystalline hexameric aggregates containing high concentrations of Zn^{2+} and Ca^{2+} ions (Howell, 1974; Havu et al., 1977). The biologically active form of the hormone is a 51 amino acid residue monomer comprised of A and B chains of total molecular weight 5800. Little is known about the molecular recognition process and the biochemical signal-transducing events that take place when the insulin monomer binds to its receptor; however, it is probable that these processes require the accessibility of specific conformational states by the insulin molecule (Blundell, 1979; Chothia et al., 1983). Consequently, the self-aggregation properties and conformational behavior of monomeric insulin and higher aggregates are subjects of great importance to understanding the expression of its hormonal activity.

The R-state insulin hexamer (Scheme IB) is a new conformational variant of hexameric insulin that recently has been described by X-ray crystallography (Derewenda et al., 1989) and identified spectroscopically in solution (Wollmer et al., 1987; Thomas & Wollmer, 1989; Kaarsholm et al., 1989; Roy

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