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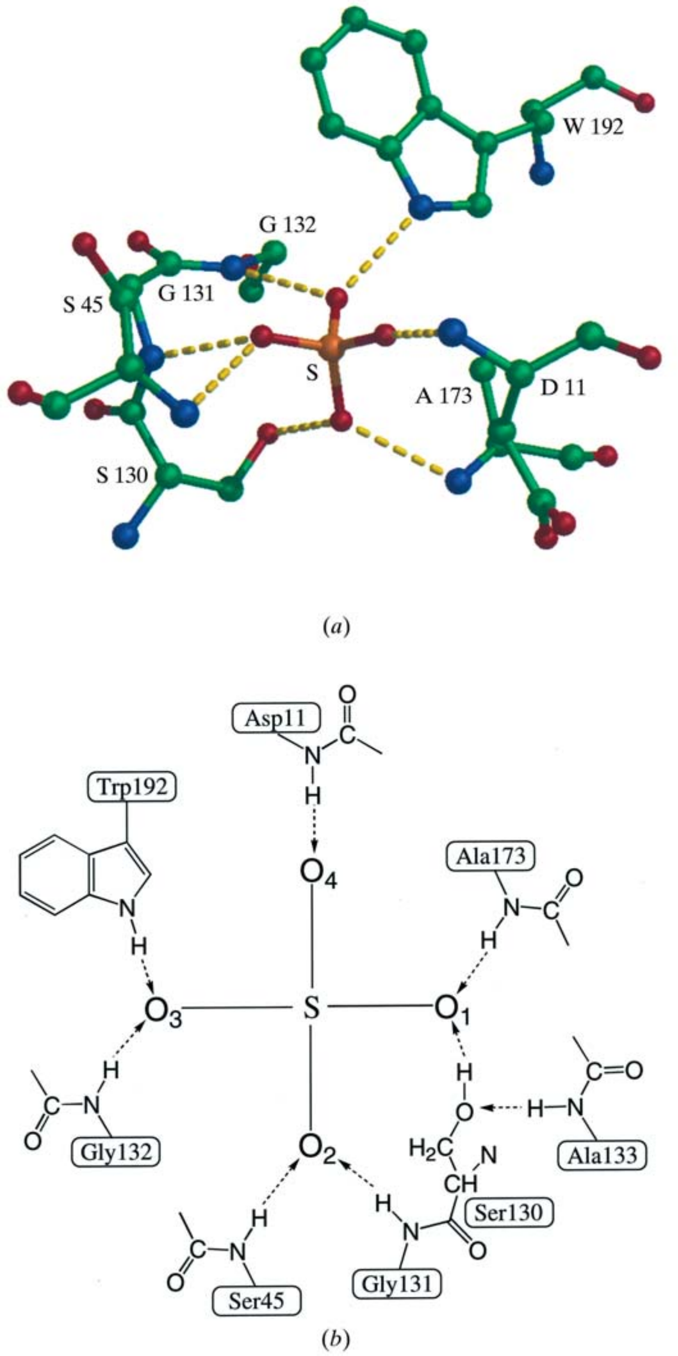

Fig. 23.2.5.2. Seven hydrogen-bonding interactions between the sulfate-binding protein (SBP) and sulfate. (*a*) Interactions based on the 1.7 Å structure (J. Sack & F. A. Quiocho, unpublished data). (*b*) Schematic diagram of the interaction.

bound sulfate (Fig. 23.2.5.2*b*) than with the bound phosphate (Fig. 23.2.5.1*b*) do not make the affinity of the SBP–sulfate complex any weaker than that of the PBP–phosphate complex. In fact, the sulfate binds 10–20 times more tightly to SBP (Pardee, 1966; Jacobson & Quiocho, 1988). Also, the hydration energies of both anions are likely to be similar.

The ability of PBP and SBP to differentiate each oxyanion ligand through the presence or absence of proton(s) is an extremely high level of sophistication in molecular recognition. The importance of complete hydrogen bonding in recognition of buried ligands is powerfully demonstrated in PBP and SBP. As the sulfate is fully ionized (*i.e.* possesses no hydrogen at physiological pH), repulsion occurs at Asp56 of PBP specifically for this dianion. On the other hand, SBP is unable to bind phosphate because it contains no hydrogen-bond acceptor in the binding site. Significantly, despite the potential for a large number of matched hydrogen-bonding pairs, a single mismatched hydrogen bond (*e.g.* a fully ionized sulfate providing no proton for interaction with Asp56 of PBP and no acceptor group in SBP for a phosphate proton) represents a binding energy barrier of 6–7 kcal mol^{-1} (1 kcal mol^{-1} = 4.184 kJ mol^{-1}).

23.2.5.1. *Dominant role of local dipoles in stabilization of isolated charges*

A novel finding of further paramount importance and wide implication is how the isolated charges of the protein-bound phosphate and sulfate are stabilized. No counter-charged residues or cations are associated with the sulfate completely buried in SBP. Although a salt link involving Arg135 is formed with the phosphate bound to PBP, it is shared with an Asp residue (Fig. 23.2.5.1*b*). Moreover, site-directed mutagenesis studies indicate that phosphate binding is quite insensitive to modulation of the salt link (Yao *et al.*, 1996). These findings are a powerful demonstration of how a protein is able to stabilize the charges by means other than salt links. Experimental and computational studies indicate that local dipoles, including the hydrogen-bonding groups and the backbone NH groups from the first turn of helices, immediately surrounding the sulfate and phosphate are responsible for charge stabilization (Pflugrath & Quiocho, 1985; Quiocho *et al.*, 1987; Åqvist *et al.*, 1991; He & Quiocho, 1993; Yao *et al.*, 1996; Ledvina *et al.*, 1996). Helix macrodipoles play little or no role in charge stabilization of the anions. The same principle of charge stabilization by local dipoles also applies for the following buried uncompensated ionic groups: Arg151 of the arabinose-binding protein (Quiocho *et al.*, 1987), the zwitterionic leucine ligand bound to the leucine/isoleucine/valine-binding protein (Quiocho *et al.*, 1987), the potassium in the pore of the potassium channel (Doyle *et al.*, 1998) and Arg56 of synaptobrevin-II in a SNARE complex (Sutton *et al.*, 1998).

23.2.5.2. *Short hydrogen bonds*

The ultra high resolution refined structure of the PBP–phosphate complex is the first to show structurally the formation of an extremely short hydrogen bond (2.432 Å) between the Asp56 carboxylate of PBP and phosphate. Although this short hydrogen bond is within the proposed range of low-barrier hydrogen bonds with estimated energies of 12–24 kcal mol^{-1} (Hibbert & Emsley, 1990), its contribution to phosphate binding affinity has been assessed to be no better than that of a normal hydrogen bond (Wang *et al.*, 1997). Thus, a unique role for short hydrogen bonds in biological systems, such as in enzyme catalysis (Gerlt & Gassman, 1993; Cleland & Kreevoy, 1994), remains controversial.

23.2.5.3. *Non-complementary negative electrostatic surface potential of protein sites specific for anions*

The presence of an uncompensated negatively charged Asp56 is unusual for an anion-binding site, as observed in PBP. In fact, a related discovery of profound ramification is that the binding-cleft region of PBP has an intense negative electrostatic surface potential (Fig. 23.2.5.3*a*) (Ledvina *et al.*, 1996). Non-complementarity between the surface potential of a binding region and an anion ligand is not unique to PBP. We have reported similar findings for SBP, a DNA-binding protein, and, even more dramatically, for the redox protein flavodoxin (Fig. 23.2.5.3*b*) (Ledvina *et al.*, 1996). Evidently, for proteins such as these, which rely on hydrogen-

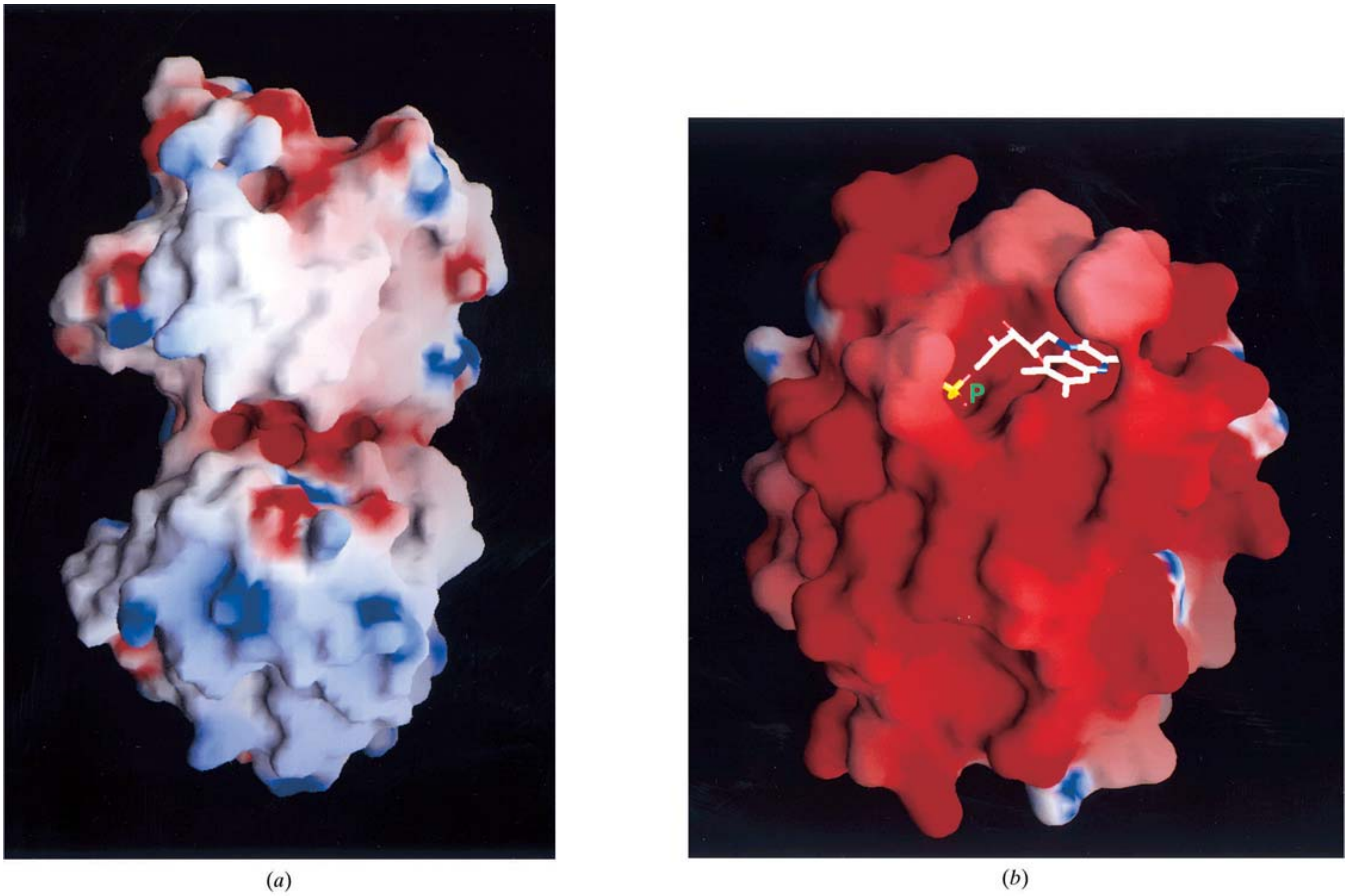

Fig. 23.2.5.3. Electrostatic surface potential of (*a*) the phosphate-binding protein and (*b*) flavodoxin. The molecular surface electrostatic potentials, calculated and displayed using *GRASP* (Nicholls *et al.*, 1991), are −10 *kT* (red), neutral (white) and +10 *kT* (blue) [see Ledvina *et al.* (1996) for more details]. (*a*) Wild-type phosphate-binding protein based on the X-ray structure of the open cleft, unliganded form (Ledvina *et al.*, 1996). The phosphate-binding site is located in the cleft (with negative surface potential) in the middle of the molecule and between the two domains. (*b*) Flavodoxin with bound flavin mononucleotide (FMN). The phosphoryl group (P) of the FMN is bound in a pocket with intense negatively charge surface potential. The surface potential was calculated without the bound flavin mononucleotide using the structure from the Protein Data Bank (PDB code: 2fox).

bonding interactions with only uncharged polar residues for anion binding and electrostatic balance, a non-complementary surface potential is not a barrier to binding. This conclusion is supported by very recent fast kinetic studies of binding of phosphate to PBP and the effect of ionic strength on binding (Ledvina *et al.*, 1998).

Acknowledgements

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references