6.2 Dihydrofolate Reductase–Trimethoprim

A classic example of a drug that works by species-specific protein inhibition is **trimethoprim** (**TMP**). Because this drug binds to bacterial dihydrofolate reductase (DHFR) $\sim 10^4$ more tightly than to the mammalian enzyme, there is a therapeutic concentration in which the drug can be used as an antibacterial with little deleterious consequences for a mammalian host.

DHFR was the first example where one has solved the X-ray crystal structure of the enzyme protein complexes for both bacteria and mammalian enzymes. Matthews et al. (56) have suggested that it is a key hydrogen bond involving the pyrimidine ring of TMP, which is present in the bacterial but not mammalian enzyme complex, that is responsible for the selectivity. This has not been definitively established with carboxyclic analogs, but analogs have clearly shown an important role of the three methoxy groups in TMP in causing species selectivity. For example, the TMP analog without the three OCH_3 groups have a binding preference for the bacterial enzyme of only ~ 10.

Kuyper (57) has analyzed the structure of the bacterial and mammalian complexes and suggested that the oxygens of the —OCH_3 group plays a key role in species selectivity. The methoxy oxygens are significantly more solvent exposed in the bacterial complex that the mammalian. Thus, because these oxygens do not form hydrogen bonds to enzyme groups in either complex, the desolvation penalty for the oxygen is smaller in the bacterial enzyme and does not as extensively cancel the favorable hydrophobic1 dispersion effects on binding of the methoxy methyl groups. This interpretation is supported by the fact that replacing the —OCH_3 with CH_2CH_3 makes the molecules less species selective; such analogs bind only a little better to bacterial DHFR but significantly better to mammalian DHFR (58, 59).

Free energy calculations/molecular dynamics have and will continue to give interesting insight into the DHFR–TMP species selectivity (39–41).

6.3 Nucleotide Intercalator

Because our first two examples have emphasized protein–small molecule interactions, we turn to a nucleic acid–small molecule interaction for our last example. There have been many experimental studies of the "intercalation" of flat, planar dyes into double-stranded DNA and other polynucleotides.

The flexibility of the sugar-phosphate backbone allows the intercalator to be sandwiched between the nucleotides with relatively little "strain." The interaction with polynucleotides by a wide variety of intercalators has been studied by physicochemical techniques. The driving force for association can be primarily hydrophobic, as in actinomycin D, where the driving force for association is AS" (57), or it can contain a large contribution from electrostatic effects as in ethidium bromide and adriamycin analogs, where the driving force for association is AH" (60) (Table 4.6). Both molecules have binding association constants K_{as} to DNA of about 10^6. The role of dispersion binding is not clear at this point, but it is likely to be very important as well (13). As noted above, the ability of these drugs to interfere with DNA replication is apparently related to their rate of dissociation k_r from DNA rather than to their association constant K_{as}. Muller and Crothers (2) showed that both actinomycin and actinomine had values of K_{as} similar to that of DNA, but the former had a much smaller k_r and a much greater effect on the rate of DNA replication. ▪

7 SUMMARY

The foregoing examples illustrate the likely nature of drug-receptor binding. It seems that hydrophobic and dispersion binding do contribute a substantial amount to the net binding affinity. However we have noted some cases (e.g., the ureido group in biotin and the intercalation of

Table 4.6 Thermodynamics of Binding of Drugs to DNA

Drug[a]	AH° (kcal/mol)	ΔS° (eu)	ΔG° (kcal/mol)
Proflavin	–6.7	+4.7	–8.1
Ethidium bromide	–6.2	+9.4	–9.0
Actinomycin D	+2.0	+39.0	–9.6
Daunomycin	–6.5	+7.7	–8.8

[a]Conditions in all cases as follow: T = 25°, 0.01 M buffer, pH = 7, 1 = 0.015 [see Quadrifoglio and Crescenzi (60)].