Molecular Recognition in Aqueous Solution: An Estimate of the Intrinsic Binding Energy of an Amide–Hydroxy Hydrogen Bond

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The intrinsic binding energy of an amide–hydroxy hydrogen bond [NHCO···HO] has been estimated (*ca.* − 12 kJ mol^{-1} at 293 K); the major contribution to this value appears to be entropic, hydrogen bond formation being driven by the release of ordered water from the carbonyl group.

In an effort to develop a general strategy for predicting the stability of a given biological interaction in aqueous solution, we have determined the intrinsic binding energy (ΔG_i)† [1,2] for hydrogen bond formation between the carbonyl group of an amide and an hydroxy group (Fig. 1). The [NHCO···HO] hydrogen bond is an important element of protein architecture.[3]

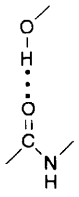


Fig. 1 Hydrogen bond formation between amide carbonyl and hydroxy groups, showing the linear geometry of the interaction relevant to our experimental system

† Defined as the Gibbs free energy change accompanying the interaction between two species in which: (*i*) there is perfect complementarity; (*ii*) there is an absence of strain; (*iii*) losses in transitional and rotational free energy have been accounted for.[1,2] It is assumed that for the interactions discussed in this paper criteria (*i*) and (*ii*) hold good. For interactions in which they do not, the term apparent binding energy[2] should be used.

The intrinsic binding energy for this hydrogen bond, ΔG_i(NHCO···HO), was determined by comparing the stability constants (K) for the separate 1:1 interactions of D-lactate (lithium salt) and *N*-Ac-D-Ala with the antibiotic ristocetin A in aqueous solution at pH 7, 293 K. It was assumed,‡ on the basis of ^{1}H NMR evidence[4] and by analogy with the 1:1 interaction of *N*-Ac-D-Ala-D-Ala with ristocetin A,[6] that both ligands bound to the antibiotic as shown in Fig.

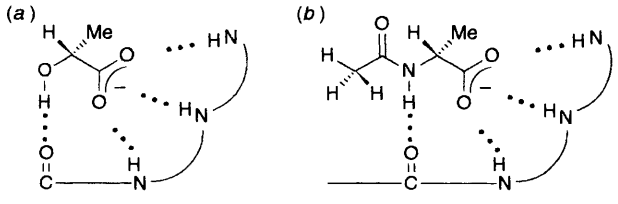
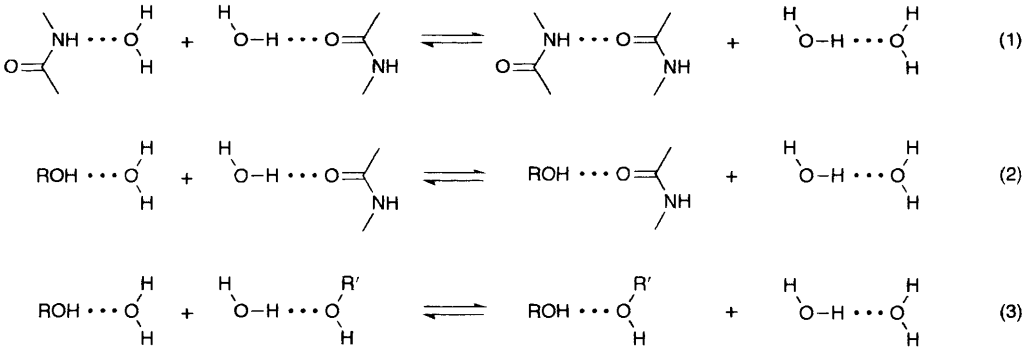


Fig. 2 Schematic representation of (*a*) D-lactate and (*b*) *N*-Ac-D-Ala interacting with ristocetin A. In addition to the four hydrogen bonds indicated, the methyl groups of both ligands form hydrophobic interactions with the aromatic ring of residue 4 of the antibiotic.[4] Another interaction, absent in the D-lactate case, is made by the two hydrogen atoms of the acetyl methyl group of *N*-Ac-D-Ala with ristocetin A.

‡ The weak interaction (Table 1) between D-lactate and ristocetin A precluded 2D ^{1}H NMR structural determinations.



Scheme 1 Equilibria for (1) amide–amide, (2) hydroxy–carbonyl and (3) hydroxy–hydroxy hydrogen bond formation. Although in reality several water molecules are likely to be ordered (relative to bulk water) by a given functionality on the left hand side of these equilibria, only one is depicted for clarity.

Table 1 Thermodynamic parameters for the interactions of D-lactate and *N*-Ac-D-Ala with ristocetin A at 293 K[a]

	K/dm^3 mol^{-1}	ΔG/kJ mol^{-1}	ΔH/kJ mol^{-1}	$T\Delta S$/kJ mol^{-1}
N-Ac-D-Ala	1032 ± 282	−16.9 ± 1.0	−32 ± 5.0	−15 ± 6.0
D-Lactate	50 ± 20	−9.5 ± 1.0	−32 ± 13	−23 ± 14

[a] Stability constants were measured at pH 7 [KH_2PO_4 (0.05 mol dm^{-3})–NaOH (0.029 mol dm^{-3}) buffer], 293 K, I = 0.1 mol dm^{-3}; complexation was monitored at 250 nm (D-lactate) and 287 nm (*N*-Ac-D-Ala). The concentration of ristocetin A varied between 0.12 and 0.15 mmol dm^{-3}. Enthalpy changes for the interactions were derived from the gradients of van't Hoff plots of $1/T$ against ln K. In the case of D-lactate, the gradient was measured between 278 and 293 K, and in the case of *N*-Ac-D-Ala between 279 and 314 K.

2. Stability constants and enthalpy changes for the interactions (Table 1) were measured by UV spectrophotometry as described in detail elsewhere.[4] Entropy changes (see also Table 1) were estimated by combining these two values, and thus carry the errors for both.

The free energy change for the interaction of *N*-Ac-D-Ala with ristocetin A relative to D-lactate is more favourable by *ca.* 8 kJ mol^{-1} (Table 1). This difference may be attributed to: (*i*) the additional translational and rotational entropy lost by *N*-Ac-D-Ala (*ca.* 4 kJ mol^{-1}) on complexation with ristocetin A;[4] (*ii*) the larger entropic loss on freezing out the internal rotation about the NH–C_α bond in *N*-Ac-D-Ala (*ca.* 5 kJ mol^{-1}) as opposed to *ca.* 3 kJ mol^{-1} lost on freezing out the internal rotation about the HO–C_α bond in D-lactate;[4] (*iii*) the hydrophobic contact made by two hydrogen atoms of the *N*-Ac-D-Ala acetyl methyl group with the antibiotic (contributing *ca.* 2 kJ mol^{-1} to the interaction);[4] (*iv*) the intrinsic binding energy of the hydrogen bond made to the carbonyl oxygen atom of the antibiotic in each case, ΔG_i(NHCO···HO) and ΔG_i(NHCO···HNCO). Earlier studies[4,6] have found ΔG_i(NHCO···HNCO) to be *ca.* −24 kJ mol^{-1} at 293 K.

Point (*iii*) contributes favourably to the 8 kJ mol^{-1} difference in binding energies, while points (*i*) and (*ii*) contribute unfavourably. An inventory of these contributions gives ΔG_i(NHCO···HO) *ca.* −12 kJ mol^{-1} at 293 K, half that for ΔG_i(NHCO···HNCO).

The entropic (ΔS_i) and enthalpic (ΔH_i) components of ΔG_i(NHCO···HO) may be inferred using a similar approach. The difference in $T\Delta S$ for the two ligand–antibiotic interactions benefits *N*-Ac-D-Ala by *ca.* 8 kJ mol^{-1} (Table 1). As points (*i*)–(*iii*) above pertain to considerations which are largely entropic[4] [again with points (*i*) and (*ii*) contributing unfavourably to $\Delta(T\Delta S)$ and point (*i*) contributing favourably], and as $T\Delta S_i$(NHCO···HNCO) = *ca.* 23 kJ mol^{-1} (ref. 4), then $T\Delta S_i$(NHCO···HO) = *ca.* 11 kJ mol^{-1} at 293 K. Similarly, the difference in ΔH for the two ligand–antibiotic interactions is, within experimental error, approximately zero (Table 1); since ΔH_i(NHCO···HNCO) has been estimated to be approximately zero,[4] and assuming that the only enthalpic difference between the two interactions would be due to the different hydrogen bonds made to the antibiotic carbonyl oxygen, then ΔH_i(NHCO···HO) may also be approximated to zero.

Although there are large errors associated with ΔH and $T\Delta S$ for the D-lactate–ristocetin A interaction, the intrinsic thermodynamic parameters derived from them for (NHCO···HO) hydrogen bond formation may be treated to a tentative physical interpretation as follows. Consider the equilibria (1)–(3) (Scheme 1). Hydrogen bond formation between two amide groups [equilibrium (1)], for which, generally[4,6] ΔG_i = *ca.* − 24 kJ mol^{-1}, ΔH_i = *ca.* − 1 kJ mol^{-1} and $T\Delta S_i$ = *ca.* 23 kJ mol^{-1} at 293 K, can be considered as being entropy-driven at this temperature. This entropy change may be associated with the release of ordered water from the amide groups when they form the (NHCO···HNCO) hydrogen bond. However, in equilibrium (2), the extent to which the hydroxy group of the alcohol ROH orders water molecules about it may be considered similar, owing to the like nature of H_2O and ROH (for instance, the dipole moments of H_2O, MeOH and EtOH are 1.85, 1.71 and 1.68 Debye respectively[7]), to the ordering of water molecules in bulk solution [see right hand side of equilibrium (2)]. Likewise the strengths of the ROH···OH_2 and H_2O···HOH hydrogen bonds might be expected to be approximately equal. Thus on going from ROH···OH_2 to H_2O···HOH, it might be reasonable to suppose that any enthalpy or entropy changes associated with hydrogen bond formation–breakage are negligible. Those that do occur on (NHCO···HO) hydrogen bond formation must therefore be due largely to the presence of the amide group. If the CO and NH dipoles of this group order water molecules to a similar extent, we might then expect ΔG_i(NHCO···HO) to be half that of ΔG_i(NHCO···HNCO), the intrinsic binding constant for amide–amide hydrogen bond formation. As ΔG_i(NHCO···HNCO) = *ca.* −24 kJ mol^{-1} and ΔG_i-(NHCO···HO) = *ca.* −12 kJ mol^{-1}, this would indeed seem to be the case. Extending the argument further, we might expect that ΔG_i for hydrogen bond formation between the hydroxy groups of two alcohols, ROH and R'OH [equilibrium (3)], to be close to zero since, if R and R' are similar, the ordering of water molecules due to the hydroxy groups of the four equilibrium species might be expected to be similar, as might the hydrogen bond strengths involved in each. Any enthalpy and entropy changes for this equilibrium would, from this argument, be negligible. Indeed the apparent binding energy†[2] of the hydrogen bond between the hydroxy group of tyrosine-34 of tyrosyl tRNA synthetase (TyrTS) and the hydroxy group of tyrosine (Tyr) in the TyrTS–Tyr complex is small (2.2 kJ mol^{-1} at 298 K).[8–10]

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