Relation between the Activity of Catalytic Antibodies and the Properties of Substrates

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The free energy of binding between catalytic antibodies and substrates is correlated with the properties of the substrates, both in the transition state (TS) and the ground state (GS). The free energy of binding was calculated from the kinetic parameters and four properties of the substrates; the solvent accessible surface area (SAS), enthalpy of hydration (ΔH), polarizability (α), and dipole moment (μ) were calculated using AM1. As a whole, TS shows a higher correlation than does GS, which is compatible with the fundamental concept of a catalytic antibody. Among these four properties, SAS gives the highest correlation coefficient (0.7616) while μ has a small correlation. Antibody-TS complexes seem to be stabilized by van der Waals force rather than a hydrophobic interaction.

Since a catalytic antibody (Ab) is produced by immunization with a hapten (an analogue of a transition state substrate-(TS)), there must be complementarity between the structure of a binding site on Ab and the structure of TS. As far as we know, however, there has been no report concerning the correlation between the physicochemical properties of TS and the binding stability. It seems reasonable to think that the structure of the binding sites on Ab reflects the properties of TS, and thus, the binding stability should be correlated with the properties of TS. This is the point that we wish to discuss in this article.

Calculation

We selected a hydrolytic reaction as a typical antibody-catalyzed reaction and analyzed the correlation between the binding free energy of the Ab–TS complex and the properties of TS calculated by a semi-empirical molecular-orbital method. The kinetic data for 22 substrates were cited from references, and the free energy of binding (ΔG_{TS}) between Ab and TS was calculated from Eq. 1,[1,2)]

$$\Delta G_{\mathrm{TS}} = -RT\ln\left(k_{\mathrm{cat}}/K_{\mathrm{m}}k_{\mathrm{uncat}}\right), \tag{1}$$

where k_{cat}, K_{m}, and k_{uncat} are the reaction-rate constant in the presence of Ab, the Michaelis constant, and the reaction-rate constant in the absence of Ab, respectively. The free energy of binding (ΔG_{GS}) between Ab and the ground-state substrate (GS) was obtained from

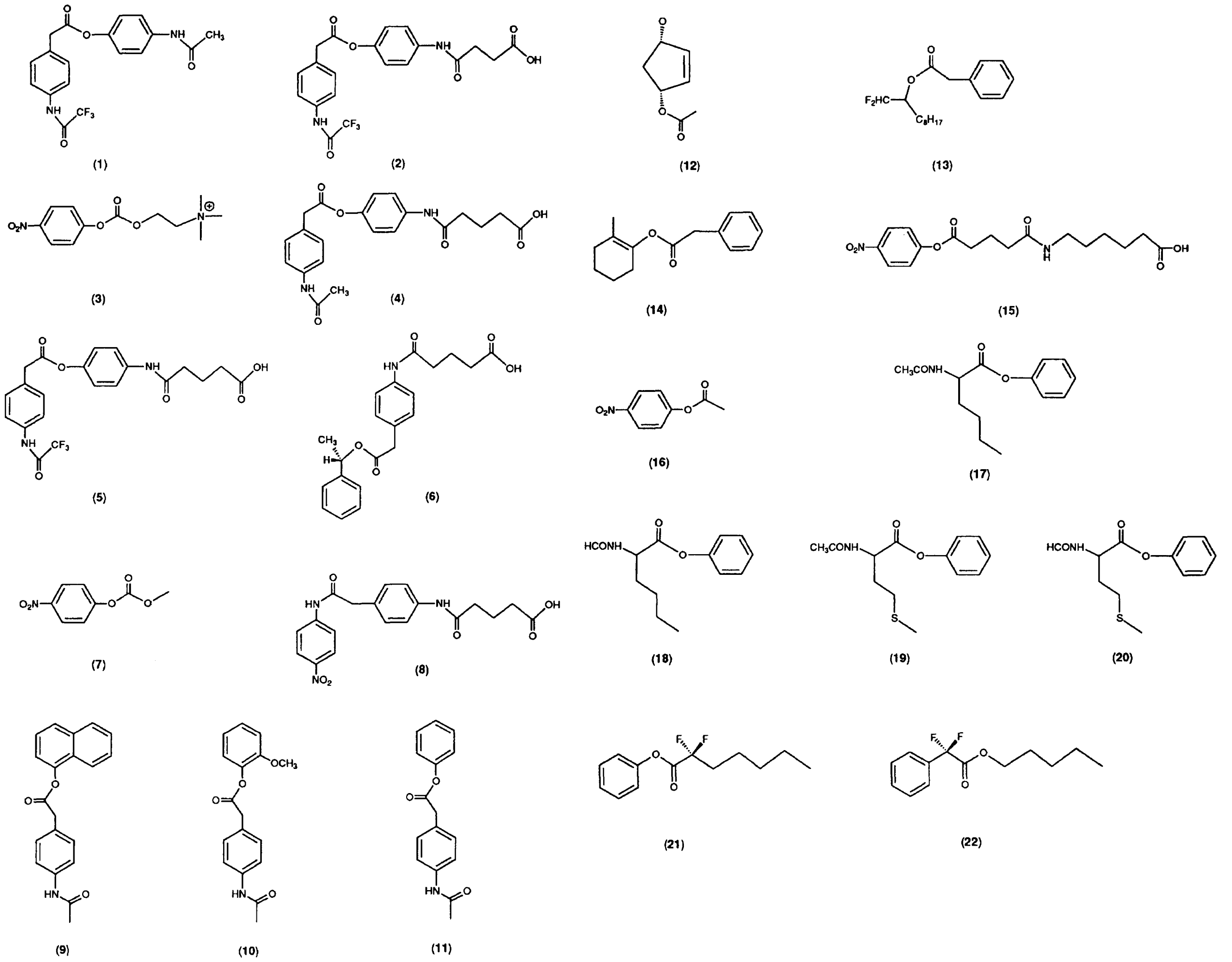
$$\Delta G_{\mathrm{GS}} = -RT\ln\left(1/K_{\mathrm{m}}\right). \tag{2}$$

Four properties of the substrates (solvent accessible surface area (SAS), enthalpy of hydration (ΔH), polarizability (α), and dipole moment (μ)) were calculated by AM1 (CAChe ver.3.6, SONY Tektronix Co.). Since the structure of the transition state in an ester hydrolysis is known to be very close to that of tetrahedral intermediate anion, we used the structure of the intermediate instead of the true transition state.

We now give a concrete method to make a calculation using the ground-state of substrate **1** (Scheme 1) as an example. The ground-state structure of **1** was first optimized by MM2 using the conjugate gradient method until convergence to 0.01 kcal mol^{-1}. Using this structure, a 1 SCF calculation was performed by AM1. Quantities α (32.47 Å^3) and μ (4.60 debye) were obtained at the same time by an AM1 calculation in vacuo. The SAS (182.09 Å^2) were also calculated by an AM1 calculation in water, where the effect of a solvent was taken into account using the CAChe optional function, COSMO (Conductor-like Screening Model).[3)] COSMO is a part of the continuum solvent model; in this study default parameters (dielectric constant 78.40; effective radius of solvent 1.00 Å) were used for water. The value of ΔH (-38.14 kcal mol^{-1}) was obtained from the difference in the heat of formation in water (-273.307 kcal mol^{-1}) and in vacuo (-235.163 kcal mol^{-1}), which were also calculated with AM1.

Results and Discussion

The free energy of binding (ΔG_{TS} and ΔG_{GS}) was calculated using Eqs. 1 and 2 and the experimental kinetic data for 22 substrates (21 esters and one amide) cited from references.[4—18)]Table 1 summarizes ΔG_{TS} and ΔG_{GS}, SAS, ΔH, α, and μ. Here, we show the reliability of the AM1 calculation by a comparison between the experimental and calculated values of α and μ for some simple compounds. For example, the experimental α values are 5.39, 6.10, 7.50 and 8.21 for *N*-methyacetamide, ethyl acetate, phenol, and aniline, resepectively.[19)] The corresponding calculated values



Scheme 1. Structures of substrates (**1**—**22**) in ground states.

Table 1. Observed Free Energy of Binding and Calculated Properties of Substrates

	Transition state					Ground State					
Substrate	ΔG_{TS}/kcal mol^{-1}	SAS/Å^2	ΔH/kcal mol^{-1}	α/Å^3	μ/Debye	ΔG_{GS}/kcal mol^{-1}	SAS/Å^2	ΔH/kcal mol^{-1}	α/Å^3	μ/Debye	Ref.
1	−12.06	169.85	−95.26	31.45	3.68	−7.92	182.09	−38.14	32.47	4.60	3,4
2	−11.82	191.04	−102.08	34.94	5.17	−8.58	187.78	−36.47	33.16	3.70	3,4
3	−9.12	132.62	−81.48	22.36	29.14	−5.10	129.25	−77.00	20.74	21.12	5
4	−13.32	188.43	−110.75	34.94	6.26	−3.90	186.13	−40.54	33.46	3.00	6
5	−12.30	197.37	−104.95	35.89	8.90	−5.28	194.45	−37.76	34.16	5.80	6
6	−10.14	180.65	−100.68	31.65	13.97	−3.30	177.88	−32.72	30.86	4.65	7
7	−8.46	99.75	−87.51	16.07	3.99	−4.38	97.76	−21.16	14.80	6.49	8
8	−12.00	178.07	−102.35	34.35	22.24	−4.50	177.70	−44.65	32.94	12.31	9
9	−8.88	155.10	−90.48	31.94	8.41	−4.32	152.87	−22.24	30.69	3.22	10
10	−8.76	146.51	−91.65	27.33	10.43	−6.18	146.09	−25.17	26.44	2.44	10
11	−8.34	138.05	−92.01	25.09	10.92	−5.34	136.71	−22.31	24.01	3.88	10
12	−7.92	101.23	−99.75	13.58	15.82	−5.22	98.48	−18.25	12.91	3.29	11
13	−8.82	157.28	−73.21	24.00	10.10	−4.68	151.62	−13.63	23.19	3.82	12
14	−7.92	120.84	−80.22	21.06	5.06	−4.32	118.04	−11.67	19.83	1.16	13
15	−10.80	178.57	−104.17	29.29	28.19	−5.64	176.26	−40.61	27.40	6.81	14
16	−8.04	95.43	−87.67	15.54	5.58	−3.36	93.21	−19.88	14.14	6.87	15
17	−10.53	130.92	−82.95	20.92	4.08	−5.07	131.68	−16.06	20.40	1.74	16
18	−10.63	124.24	−83.00	19.70	3.69	−4.96	123.88	−17.33	19.17	1.86	16
19	−8.63	136.28	−86.78	21.98	4.38	−4.13	136.10	−20.87	21.35	1.46	16
20	−9.23	128.61	−86.83	20.75	3.02	−3.65	128.61	−22.27	20.11	1.05	16
21	−10.23	124.78	−81.10	18.92	7.79	−4.93	124.20	−9.05	18.27	0.62	17
22	−10.30	125.46	−82.55	18.06	6.27	−4.85	123.18	−9.49	17.61	0.81	17
r		0.7616	0.5958	0.7060	0.0224		0.3846	0.2215	0.3518	0.0148	

are 5.13, 5.97, 7.98, and 8.64. The experimental μ values are, respectively, 4.39, 1.88, 1.45, and 1.51,[19)] while the calculated values are 3.50, 1.79, 1.23, and 1.54. It therefore seems that as a whole an AM1 calculation gives relatively good results for α and μ. As for SAS, we could not check the reliability, since no experimental data were available. How-

ever, since this quantity is simply determined by the shape and size of molecules, the calculated data must be fairly correct. On the other hand, ΔH calculated using the continuum solvent model is expected to have some deviation from the experimental values. Actually, the experimental ΔH values are -5.0 and -12.7 kcal mol^{-1} for acetaldehyde and benzoic acid,[19] though the calculated values are -9.39 and -15.85 kcal mol^{-1}. Thus, the calculated ΔH should be regarded as being an indicator which shows the relative degree of hydration. The correlation coefficient (r) between ΔG_{TS} or ΔG_{GS} and the individual property is also given at the bottom of Table 1. The most important result to be noted here is that r of TS is higher than that of GS, which is compatible with the fundamental concept that Ab recognizes TS rather than GS. Among the four properties of TS, SAS gave the highest r value (0.7616). Figure 1 shows the relation between the free energy of binding and SAS, where as a whole TS with larger SAS tends to form more stable complexes with Ab (the slope of the plot is ca. -40 cal mol^{-1} Å^{-2}). This result seems to be reasonable since molecules with a larger SAS tend to possess more binding sites recognized by Ab. Figure 2 also shows the relation between the free energy of binding and α, where TS with larger α is apt to form more stable complexes. This finding suggests that the main interaction between Ab and TS is van der Waals force. Similarly, Fig. 3 illustrates the relation between the free energy of binding and ΔH, where TS with a more negative ΔH can form more stable complexes. This also indicates that the complexes between Ab and TS are mainly stabilized by van der Waals force rather than a hydrophobic interaction. Therefore, one of the methods to increase the activity ($k_{\mathrm{cat}}/K_{\mathrm{m}}k_{\mathrm{uncat}}$ value) of catalytic antibodies may be to introduce many polar groups (such as amide and ester groups) into the substrates. Contrary to the above three quantities, μ gave a very low r value, probably because it shows only the macroscopic character of a whole molecule, and does not reflect the local environments. On the other hand, SAS, ΔH and α include more detailed information about the local structures and electronic states, and, consequently, have a higher correlation with ΔG_{TS}.

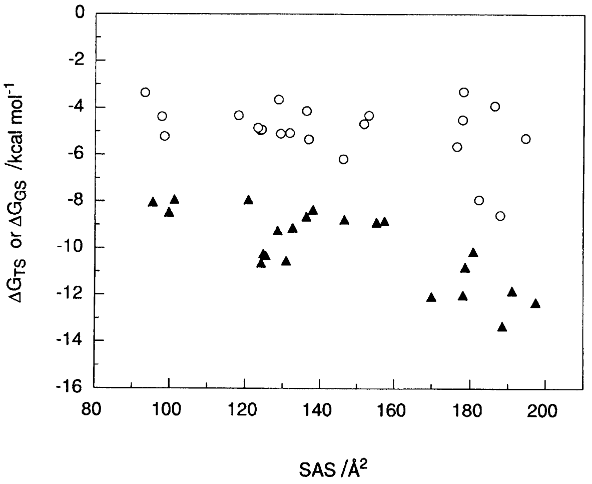


Fig. 1. Relation between free energy of binding (ΔG_{TS} or ΔG_{GS}) and solvent accessible surface area (SAS); (▲) transition state, (○) ground state.

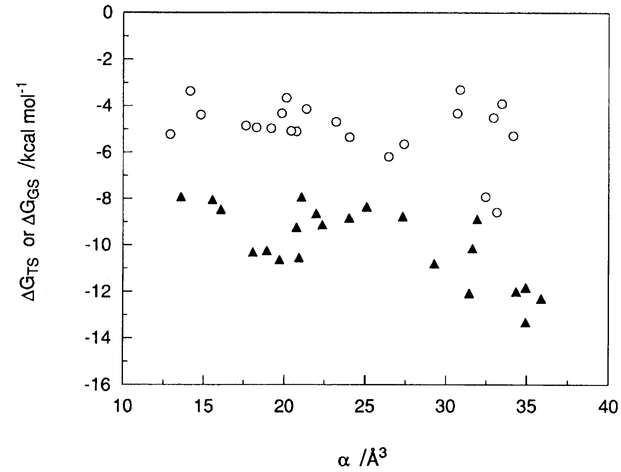


Fig. 2. Relation between free energy of binding (ΔG_{TS} or ΔG_{GS}) and polarizability (α); (▲) transition state, (○) ground state.

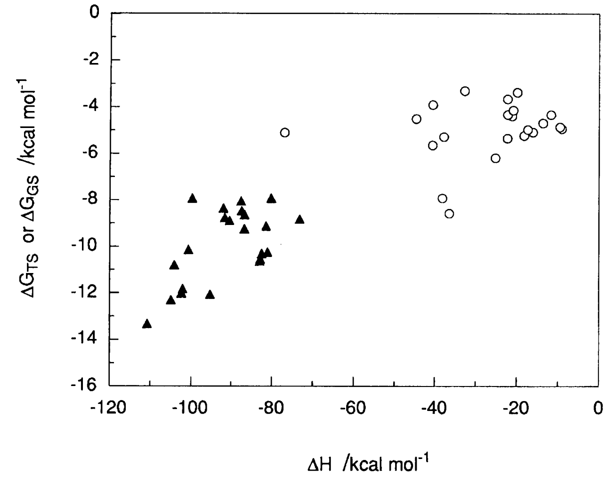


Fig. 3. Relation between free energy of binding (ΔG_{TS} or ΔG_{GS}) and enthalpy of hydration (ΔH); (▲) transition state, (○) ground state.

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