Journal of Chromatography, 376 (1986) 111—119
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2946

NATURE OF THE ANTIGEN—ANTIBODY INTERACTION

PRIMARY AND SECONDARY BONDS: OPTIMAL CONDITIONS FOR ASSOCIATION AND DISSOCIATION

C.J. VAN OSS*

Departments of Microbiology and Chemical Engineering, State University of New York at Buffalo, Buffalo, NY 14214 (U.S.A.)

R.J. GOOD

Department of Chemical Engineering, State University of New York of Buffalo, Buffalo, NY 14260 (U.S.A.)

and

M.K. CHAUDHURY

Dow-Corning Corporation, Midland, MI 48640 (U.S.A.)

SUMMARY

All antigen—antibody (AG—AB) bonds are weak physical bonds; covalent bonds are not encountered. The main bonds involved are: (I) Coulombic bonds; (II) Ca^{2+}-bridges; (III) hydrogen bonds; (IV) Lifshitz—van der Waals bonds. Combinations of III and IV occur as the "bonds" usually alluded to as hydrophobic (Hϕ) interactions. In primary bonds, mainly types I and IV occur; types II and III are quite rare. Secondary bonds, which evolve after a certain time-lapse (varying from minutes to days), mainly involve type IV bonds and Hϕ interactions, while hydrogen bonds sensu stricto have been known to play a role in rare instances.

In affinity chromatography involving AG—AB interactions, complete elution with mild eluents usually is desirable. It would thus appear essential to let little time elapse between AG—AB complex formation and the elution step, to minimize strengthening of the AG—AB interaction by secondary bond formation. For expeditious elution, it also is important to avoid using dehydrating agents (which tend to decrease the bond distance between AG and AB and thus could strengthen the bond in a number of ways).

The degree of involvement of types I and IV bonds as well as of Hϕ interactions varies considerably among different AG—AB systems. These components thus have to be measured

0378-4347/86/$03.50

separately in order to determine the optimal conditions for elution. The parameters of the liquid medium that may be modulated to influence dissociation (or association) are, for example, surface tension, pH, ionic strength, dielectric constant, temperature, admixture of dehydrating agents or of chaotropic salts. The influenc of variations in each of these parameters on types I, III and IV bonds (and thus also on Hφ interactions) will be delineated. Because of the misleading implications of the term "hydrophobic interactions", it seems preferable henceforth to allude to them as "interfacial forces".

INTRODUCTION

The active research on the antigen—antibody (AG—AB) bond, which continued into the early 1970s and culminated in the books published by Kabat [1] and Pressman and Grossberg [2], has since somewhat slowed down. The principal aspect that continues to be actively explored is the nature of antigenic determinants (AGD) [3, 4] and of the antibody-active sites (AAS) that follow from them [5]. However, the actual nature of the AG—AB bond still has many aspects that remain virtually unexplored. One is the, as yet, rather vague mechanism of "hydrophobic interactions". Another aspect is the differentiation between primary and secondary AG—AB bonds. The influence of Coulombic bonds, Lifshitz—van der Waals bonds and hydrophobic interactions on primary and secondary bond formation and on conditions favoring AG—AB association and dissociation, is discussed below.

TYPES OF BONDS

From the relatively weak AG—AB bond energies, derived from equilibrium measurements [6, 7], it can be deduced that AG—AB bonds are not covalent. From the various means by which AG—AB bonds either can be prevented from forming, or can be dissociated, it transpires that these non-covalent bonds consist mainly of Coulombic and/or van der Waals bonds, although on occasion (most often in the case of secondary bonds, as is discussed below), hydrogen bonds have been shown to occur [2].

Coulombic bonds

Coulombic bonds, i.e. the bonds between oppositely charged amino acids in AGD and AAS, represent one of the two most common AG—AB bonds. Generally, when AG and AB combine into complexes via primary Coulombic bonds, due to the ensuing close proximity between AGD and AAS, van der Waals bonds also arise. However, it was found, somewhat unexpectedly, that in the cause of (auto-) ABs to double-stranded DNA, the net attractive energy of the van der Waals bonds and of the other components of the interfacial forces (see below), is negligibly small, owing to the exceptional hydrophilicity of both AGD and AAS [8]. The DNA—anti-DNA system may well be the main representative of a category of solely Coulombic AG—AB interactions in which, notwithstanding the close approach between AGD and AAS, no significant net van der Waals and/or interfacial attraction occurs.

Ca^{2+}-bridging

In various biological systems, attraction between negatively charged

particles, cells or macromolecules often are effected through cross-linking by means of plurivalent cations, usually Ca^{2+}. It could therefore be expected that among the different types of AG—AB linkages Ca^{2+} bridging also might occur. This can indeed be the case, but the occurrence is quite rare. The only known compound in this category involves the synthetic polypeptide polyglutamic acid and its AB, the complexes of which can be dissociated with EDTA [9]. Ca^{2+}-bridging has not been found to occur in the DNA- anti-DNA system [10], even though this system also involves a strongly negatively charged polyelectrocyte AG.

Hydrogen bonds

Like Coulombic bonds, hydrogen bonds are of polar origin. But whilst under optimal conditions hydrogen bonds may be stronger than Coulombic or van der Waals bonds, hydrogen bonds differ from both Coulombic and van der Waals bonds in being only operative at very short distances (of the order of 1.5—5 Å). Coulombic bonds and regular van der Waals bonds can make themselves felt at distances of the order of 100 Å, whilst long-range (or retarded) van der Waals bonds may be operative at distances of more than 1000 Å. Due to their very short range of action, and due to the fact that hydrogen bonds in aqueous media are strongly attenuated by hydrogen-bond formation between hydrogen donor and hydrogen receptor moieties and the ambient water molecules, direct hydrogen bonding is seldom encountered in primary binding between AGD and AAS. In secondary AG—AB bond formation, however, hydrogen bonds are known to occur [11, 12]. On the other hand, more indirectly, hydrogen bonds, together with Lifshitz—van der Waals bonds, play a crucial role in bonds caused by hydrophobic interactions, or, more precisely, by interfacial forces (see below).

Lifshitz—van der Waals (LW) bonds

Between all atoms that are brought very closely together, electrodynamic attractions arise between fluctuating dipoles occurring in one atom, and other dipoles induced by it in a neighboring atom, in addition to permanent dipole–dipole and permanent dipole—induced-dipole attractions.

The magnitude of LW forces between two substances can be derived from the substances' long-range (or LW) surface tension [13] and from the distance between them. When the interaction takes place in a liquid (e.g. water), the properties of the liquid must also be taken into account.

Interfacial forces (hydrophobic interactions)

The attraction between two hydrophobic (apolar) moieties in water, and also between a hydrophilic (polar) and a hydrophobic (apolar) determinant in water, is often alluded to as a hydrophobic bond. This term is misleading, as it implies that there is a unique physicochemical "bond", which might induce one to believe that its underlying physical nature is thoroughly known and that its energy may be quantitatively expressed in a simple equation. However, hydrophobic interactions of this nature have a fairly complex underlying mechanism of which one of the major contributing factors is the LW interaction [14, 15], whilst short-range interactions (i.e. hydrogen bonds) also play a crucial role in

aqueous media and should be treated separately from, and in addition to, LW bonds [13].

The three van der Waals bonds (London, Debye, Keesom) may be grouped together (as is legitimate in the liquid state) [13], and will be alluded to as long-range or LW interactions [13], whilst hydrogen bonds in aqueous media are designated as short-range (SR) interactions [13].

The energy of LW interactions (in the configuration of two plane parallel slabs) decreases inversely proportionally to the distance between the slabs, whilst that of SR interactions decays at an exponential rate, which is the reason why these interactions should be treated separately and why the latter ceases to be of much influence beyond a distance of $\approx$ 5 Å. However, at the minimum equilibrium distance, d_0 (i.e. at $d_0 \approx 1.54$ Å), between the outer electron shells of the atoms involved [16] the LW and the SR interactions may be taken as additive, if the values of these interactions have been derived from contact angle measurements, which themselves have been obtained at that distance d_0. Thus for interactions at molecular contact, the sum of the free energies of interaction of LW and SR interactions will yield the total free energy of interaction, i.e.:

$$\Delta G^{LW} + \Delta G^{SR} = \Delta G^{TOT\text{-}IF} \tag{1}$$

where $\Delta G^{TOT\text{-}IF}$ represents the total free energy of the interfacial forces, often alluded to as hydrophobic interaction [14, 15], where

$$\Delta G^{LW} = \gamma^{LW}_{AG-AB} - \gamma^{LW}_{AG-H_2O} - \gamma^{LW}_{AB-H_2O} \tag{2}$$

$$\Delta G^{SR} = \gamma^{SR}_{AG-AB} - \gamma^{SR}_{AG-H_2O} - \gamma^{SR}_{AB-H_2O} \tag{3}$$

It is stipulated here that the free energy of the electrostatic interaction of the determinant in question is either zero, or is to be determined separately; in the latter case, its value should of course be added to $\Delta G^{TOT\text{-}IF}$ to obtain ΔG^{TOT}_{AG-AB}:

$$\Delta G^{TOT\text{-}IF} + \Delta G^{COUL} = \Delta G^{TOT}_{AG-AB} \tag{4}$$

The degree of hydration of both AG and AB plays an important role in determining the values of both ΔG^{LW} and ΔG^{SR}: both ΔG^{LW} and ΔG^{SR} values tend to be low when AG and AB are strongly hydrated; both ΔG^{LW} and ΔG^{SR} tend to become more negative when AG and AB become dehydrated.

The degree of extrusion of interstitial water between AGD and AAS also strongly influences the values of ΔG^{LW} and ΔG^{SR}. Upon extrusion of interstitial water, eqns. 2 and 3 become:

$$\Delta G^{LW} = \gamma^{LW}_{AG-AB} - \gamma^{LW}_{AG} - \gamma^{LW}_{AB} \tag{5}$$

$$\Delta G^{SR} = \gamma^{SR}_{AG-AB} - \gamma^{SR}_{AG} - \gamma^{SR}_{AB} \tag{6}$$

For slightly to stronger dehydrated AGD and AAS, the progressive loss of interstitial water typically leads to a decrease in ΔG^{SR}, accompanied by a marked increase in ΔG^{LW}, often resulting in little or no change in $\Delta G^{TOT\text{-}IF}$. This phenomenon conforms well with Mukkur's important observation that, with hapten–AB interactions, an increase in temperature leads to a decrease in the

negative value of ΔH, which is compensated for by corresponding change in the value of $T\Delta S$ [17, 18]; see also ref. 12. As an increase in T tends to cause a decrease in hydrogen bonding, ΔG^{SR} then also decreases. The concomitant increase in ΔG^{LW} with T assures the relative constancy of $\Delta G^{TOT\text{-}IF}$, whilst the accompanying increase in positive entropy of the system is simply a manifestation of the increase in randomization of previously more organized interstitial water molecules, upon their extrusion into the bulk liquid. Thus, increases in T can by no means invariably be counted upon to favor the dissociation of AG—AB complexes.

As the term "hydrophobic interactions" also is misleading, since it often pertains to interactions between two hydrophilic groups and one hydrophobic group, i.e. between a hydrophilic and a hydrophobic determinant, immersed in a hydrophilic medium (water), it is proposed to designate them instead as "interfacial forces" [14, 15].

PRIMARY BONDS

Most of the specificity of the bonds that arise between AGD and AAS is due to the interactions that occur very early in the process of AG—AB binding and is the main cause of the incipient attraction between AGD and AAS while these moieties still are some distance apart. As the distance at which AGD and AAS start attracting each other to a significant degree is likely to be of the order of 100 Å for Coulombic bonds and may be 1000 Å or more for LW bonds, the very-short-range hydrogen bonds (see above) are unlikely to play a direct role in primary AGD—AAS interactions. Although the primary bond is mainly responsible for the specificity of the AGD—AAS interactions, in many cases, the primary bond energy tends to be significantly smaller than the total (primary + secondary) AG—AB bond energy.

The primary bond energy can be determined by measuring the energy required to prevent the bond from forming. A few examples of this can be

TABLE I

PRIMARY AND SECONDARY AG—AB BOND ENERGIES

See also ref. 21. COUL = coulombic bond; IF = interfacial forces.

System	Reference(s)	Energy (kcal/mol)		
		Primary bond	Secondary bond	Total bond energy
BSA—anti-BSA	18	3.3 (COUL)	7.0 (IF)	10.3
P3A—anti-P3	19	4.0 (IF)	2.8 (IF)	6.8**
DNA—anti-DNA (low affinity)	10, 11	1.3 (COUL)	0	1.3
DNA—anti-DNA (high affinity)	11	3.6 (COUL)	3.6* (hydrogen bond)	3.6

*From the published value of $K_2 = 1.6 \cdot 10^{-5}$ mol/l [22], this would be 6.2 kcal/mol, which agrees reasonably well with this computed value.

**The extent to which these Coulombic bonds convert to hydrogen bonds is not quantitatively known: the *total* bond energy appears to remain unchanged.

given. First, to prevent bovine serum albumin (BSA) from reacting with anti-BSA, it suffices to raise the pH from 7.0 to 9.5 [18]. This corresponds (if one may assume, as seems reasonable, that as a first approximation kinetic factors may be neglected) to $\Delta G^{COUL} = -RT\ln 316 = -3.3$ kcal/mol, while the total energy of formation at 20°C (for $K = 5 \cdot 10^7$ l/mol) [18] is —10.3 kcal/mol (see Table I). Second, in a purely van der Waals system, to prevent the interaction between 3-azopyridine (P3), coupled to rabbit serum albumin (P3A), with rabbit anti-P3, it suffices to lower the surface tension of the aqueous medium from 72.8 to 60 erg/cm^2 [19], yielding an energy $\Delta G^{TOT\text{-}IF} = -14$ erg/cm^2, which amounts (assuming a total surface area of AGD of 200 Å^2) to ca. —4.0 kcal/mol. The energy needed to dissociate anti-P3 from P3A requires a lowering of the surface tension of the liquid to 50 erg/cm^2 (which yields the total energy for the binding of P3A to anti-P3, amounting to $\Delta G^{TOT\text{-}IF} = -24$ erg/cm^2), which corresponds to —6.8 kcal/mol (see Table I). In purely electrostatic systems (e.g. the low- and medium-affinity DNA—anti-DNA systems [10], there is no measurable difference between the primary and the total energy of association, deduced from the equality between the energies of dissociation and of prevention of association [12]; see Table I.

SECONDARY BONDS

Hysteresis

The greater energy needed for dissociating most AG—AB bonds than is required for the prevention of their association (hysteresis) is indicative of further, secondary bonds, that have formed subsequent to the formation of the initial, primary AG—AB bonds. The difference between the energy of dissociation and the energy required to prevent association of AG from AB (hysteresis) is equal to the energy of the secondary AG—AB bonds. Thus, the energy of the secondary bond is derived as follows (see Table I) [21]:

$$\Delta G_{secondary} = \Delta G_{dissociation} - \Delta G_{prevention\ of\ association} = \Delta G_{total} - \Delta G_{primary} \quad (7)$$

Types of secondary bonds

Coulombic bonds. Coulombic bonds do not seem to occur as secondary bonds. The purely Coulombic DNA—anti-DNA system has been studied from this aspect: the similarities of pH conditions, leading to dissociation and to prevention of association (lack of hysteresis), points to an absence of secondary Coulombic bonds in this sytem [10]. Secondary Coulombic Bonds are likely to be rare occurrences, as the statistical probability of negatively and positively charged amino acids on AG and AB, outside of the AGD and AAS, being situated exactly opposite each other, is very slight, and in those rare cases where it occurs, such moieities would be indistinguishable from primary bonds.

Hydrogen bonds. Hydrogen bonds, which do not seem to play a significant role in direct primary AGD—AAS interaction, may occur in secondary AG—AB bonding, e.g. in high-avidity DNA—anti-DNA systems [11] (see Table I). Although precise binding studies have not yet been performed on this system, from preliminary data (i.e. from the pH needed to dissociate DNA—anti-DNA,

or to prevent them from associating) it would not appear that the formation of secondary hydrogen bonds causes an increase in the total binding energy [11, 12]. In this case, Coulombic bonds apparently gradually revert to hydrogen bonds, without a significant change in energy. What points to the formation of hydrogen bonds in high-avidity DNA—anti-DNA complexes, is the impossibility of dissociating them at high ionic strengths, although fairly low ionic strengths suffice to prevent them from forming at all [10, 11]. Chaotropic ions, however, which especially dissociate hydrogen bonds, do dissociate these high-avidity DNA—anti-DNA complexes [11].

Interfacial forces. Interfacial forces by their nature represent the most common bonds involved in secondary AG—AB bonding. As soon as AGD and AAS have combined in a primary bond, various non-specific (especially non-polar) moieties of AG and/or AB in the immediate vicinity of AGD and AAS can undergo a mutual interfacial attraction, approach each other more closely and bind to each other secondarily. Thus, both in cases where the primary AGD—AAS bond is solely of the interfacial type (e.g. P3A—anti-P3) and in the cases where the primary bond is mainly electrostatic (BSA—anti-BSA), secondary bonds of the interfacial type are bound to develop (see Table I). With time, a strengthening of existing (primary as well as secondary) interfacial bonds also takes place through the extrusion of interstitial solvent. This results in a shorter distance between AGD and AAS, which enhances the interfacial attraction energy and results in an (at least partial) direct AGD—AAS contact that replaces the AGD—water—AAS interaction, which gives rise to a further significant increase in the total energy of attraction [14, 15].

CONDITIONS FAVORING ASSOCIATION OR DISSOCIATION

In many cases, the conditions favoring association of AG with AB are qualitatively (and in some cases quantitatively) the inverse of the conditions favoring their dissociation. However, as shown above, when secondary bonds occur, dissociation usually requires more energy than the prevention of association, in which case a quantitative difference exists between the two opposing effects.

In general, however, as shown in Table II, when the conditions required to effect dissociation are reversed, association is favored instead. For the sake of simplicity it therefore suffices to describe only the various conditions by which dissociation of AG—AB complexes can be achieved.

In most cases, dissociation is most readily achieved by combining a decrease in the surface tension of the aqueous medium with an increase (or in some cases with a drastic decrease) in pH. Only purely Coulombic systems dissociate well by increasing the ionic strength; in systems with mainly interfacial bonding the opposite is true. However, addition of chaotropic salts favors dissociation of both interfacial and Coulombic bonds. The effect of an increase in temperature is often so multifaceted as to make its outcome difficult to predict, and indeed it often may have no net effect at all [12, 17, 18]. Decreasing the dielectric constant of the medium also may have ambiguous effects or no effect at all, depending on the proportion of interfacial and Coulombic forces in the complexes. If total dissociation is desired, it is advis-

TABLE II

CONDITIONS FAVORING (+) OR HINDERING (−) DISSOCIATION OR ASSOCIATION OF ANTIGEN–ANTIBODY COMPLEXES, BROKEN DOWN ACCORDING TO LONG-RANGE LIFSHITZ–VAN DER WAALS INTERACTIONS (ΔG^{LW}), SHORT-RANGE, i.e. MAINLY HYDROGEN BOND INTERACTIONS (ΔG^{SR}), AND ELECTROSTATIC INTERACTIONS (ΔG^{COUL})

The strongest effects are indicated by ++ or − −.

Dissociation[a]			Variable parameter	Association[a]		
ΔG^{LW}	ΔG^{SR}[b]	ΔG^{COUL}		ΔG^{LW}	ΔG^{SR}[b]	ΔG^{COUL}
− or +	++		Decrease in γ^{Total}_{Liquid}[c]	+ or −	− −	
		++	Increase or decrease in pH			− −
	−[d]	+	Increase in ionic strength		+[d]	−
−	−	− (+)[e]	Addition of dehydrating agents, e.g. $(NH_4)_2SO_4$ or polyethyleneglycol	+	+	+ (−)[e]
	++[f]	±	Additions of chaotropic agents, e.g., KCNS, guadine · HCl		− −	−
+ or −[g]	−[f]	+	Increase in temperature[a]	− or +[g]	+	−
+[h]	+[h]	−	Decrease in dielectric constant	−[h]	−[h]	+
−[i]	−[i]		Increase in time	+[i]	+[i]	
+	+	+	Addition of haptens	−	−	−

[a]The total free energy due to interfacial forces ($\Delta G^{TOT\text{-}IF}$) (hydrophobic interaction) comprises both ΔG^{LW} and ΔG^{SR} components. Thus the influence of various parameters on this interaction (especially changes in temperature) usually cannot be simply predicted in a general manner, and the separate influences on ΔG^{LW} and ΔG^{SR} need to be determined first [15, 16]. The total influence on association or dissociation of an increase in temperature often is hard to predict [12] and in many cases, may actually be negligible, due to the $\Delta H - T\Delta S$ compensation phenomenon; see ref. 17.

[b]All changes pertaining to ΔG^{SR} of course also apply to hydrogen-bonds sensu stricto.

[c]Decreasing γ^{Total}_{Liquid} usually is accompanied by a lower γ^{SR}_{Liquid} and a higher γ^{LW}_{Liquid} in aqueous media; due to secondary bond formation, a more drastic lowering of γ^{Total}_{Liquid} is required to achieve dissociation than is needed to prevent association [19].

[d]May prevent hydrogen-bonds sensu stricto from forming but will not dissociate such hydrogen bonds, once formed [11].

[e]The admixture of large amounts of neutral salts tends to cause dissociation of low- and medium-avidity Coulombic complexes [8].

[f]The same effects occurring in these two cases pertain to hydrogen-bonds sensu stricto.

[g]The second contingency prevails when an increase in temperature causes a considerable degree of extrusion of inerstitial water.

[h]Ref. 13.

[i]Mainly applicable to secondary bonds.

able to allow as little time as possible to elapse after the initial association, to minimize the formation of secondary bonds. Dissociation of an AG from AG—AB complexes by replacing it with the corresponding hapten is quite effective, but of course this is only feasible in those rare cases where the appropriate hapten happens to be available. In Table II, the effects on ΔG^{LW}, ΔG^{SR} and ΔG^{COUL} of the various changes brought about in the properties of the suspending media of AG and AB are listed.

REFERENCES

1 E.A. Kabat, Structural Concepts in Immunology and Immunochemistry, Holt, Rinehart and Winston, New York, 1976.
2 D. Pressman and A.L. Grossberg, The Structural Basis of Antibody Specificity, Benjamin, Reading, MA, 1973.
3 M.Z. Atassi, Immunochemistry, 12 (1975) 423.
4 M.Z. Atassi, in M.Z. Atassi, C.J. van Oss and D.R. Absolom (Editors), Molecular Immunology, Marcel Dekker, New York, 1984, p. 15.
5 S.S. Twining and M.Z. Atassi, J. Biol. Chem., 253 (1978) 5259.
6 C.J. van Oss and D.R. Absolom, in M.Z. Atassi, C.J. van Oss and D.R. Absolom (Editors), Molecular Immunology, Marcel Dekker, New York, 1984, p. 337.
7 C.J. van Oss and A.L. Grossberg, in N.R. Rose, F. Milgrom and C.J. van Oss (Editors), Principles of Immunology, MacMillan, New York, 1979, p. 65.
8 E.R. de Groot, M.C. Lamers, L.A. Aarden, R.J.T. Smeenk and C.J. van Oss, Immunol. Commun., 9 (1980) 515.
9 P.A. Liberti, Immunochemistry, 12 (1975) 303.
10 R.J.T. Smeenk, L.A. Aarden and C.J. van Oss, Immunol. Commun., 12 (1983) 177.
11 C.J. van Oss, R.J.T. Smeenk and L.A. Aarden, Immunol. Invest., 14 (1985) 245.
12 D.R. Absolom and C.J. van Oss, CRC Crit. Rev. Immunol., in press.
13 M.K. Chaudhury, Short Range and Long Range Forces in Colloidal and Macroscopic Systems, Ph.D. Thesis, State University of New York at Buffalo, Buffalo, 1984.
14 C.J. van Oss, R.J. Good and M.K. Chaudhury, 5th Int. Conf. Surface Colloid Sci., Clarkson University, Potsdam, NY, 1985, Abstract No. 165.
15 C.J. van Oss, R.J. Good and M.K. Chaudhury, J. Colloid Interface Sci., in press.
16 M.K. Chaudhury, R.J. Good and C.J. van Oss, in preparation.
17 T.K.S. Mukkur, CRC Crit. Rev. Biochem., 16 (1984) 133.
18 C.J. van Oss, D.R. Absolom and P.M. Bronson, Immunol. Commun., 11 (1982) 139.
19 C.J. van Oss, D.R. Absolom, A.L. Grossberg and A.W. Neumann, Immunol. Commun., 8 (1979) 11.
20 C.J. van Oss, D.R. Absolom and A.W. Neumann, in T.C.J. Gribnau, J. Visser and R.J.F. Nivard (Editors), Affinity Chromatography and Related Techniques, Elsevier, Amsterdam, 1982.
21 C.J. van Oss and D.R. Absolom, Convocation Immunol., 8 (1986) 67.
22 A.L. Grossberg, G. Radzinksy and D. Pressman, Biochem. J., 1 (1962) 391.