



Human monoclonal antibody stability and activity at vaginal pH

Philip E. Castle ^{a,*}, Daniel A. Karp ^a, Larry Zeitlin ^{a,b},
Bertrand García-Moreno E. ^a, Thomas R. Moench ^c,
Kevin J. Whaley ^{a,b,c}, Richard A. Cone ^{a,c}

^a Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218, USA

^b EPIcyte, Inc, San Diego, CA, USA

^c ReProtect, LLC, Baltimore, MD, USA

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Abstract

Antibodies can be delivered topically to the vagina to protect against pregnancy and sexually transmitted infections, but the acidity of vaginal secretions (pH 3.5–4.5) might inactivate them. To address this question, both experimental and computational methods were used to evaluate the effects of pH on human monoclonal antibody (MAb) stability and activity. To determine the acid-sensitivity of their antigen binding sites, human MAbs against human sperm (H6-3C4) and gp120 of HIV (1511) were tested by ELISA for binding to human sperm and recombinant gp120, respectively, at pH 3.0–7.0, after storing them for 1 or 20 h at the same pH. Binding was unaltered by acidic pH ≥ 4 even after 20 h, and at pH 3.5 both MAbs retained $\geq 40\%$ antigen binding activity. A humanized MAb against HSV-2 glycoprotein B expressed both in Chinese hamster ovary (CHO) cells and in soybean cells was incubated for 1 or 24 h at pH 3.5–7.6, brought to neutral pH, and tested for ability to block HSV-2 infection of foreskin fibroblast cells. Loss in blocking activity occurred only when antibodies were incubated at pH 3.5 for 24 h and was independent of the expression cell type. Using empirical structure-based methods, net charge, Z , and electrostatic contributions to free energy, $\Delta\Delta G_{ei}$, were calculated as a function of pH for 1 human and 8 murine

* Corresponding author. Present address: Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH 6120 Executive Blvd., Room 7074 Rockville, MD 20892-7234, USA. Tel.: +1-301-435-3976; fax: +1-301-402-0916.

E-mail address: pc95p@nih.gov (P.E. Castle).

F(ab)s. The calculations indicate that Z changes slowly between pH 5.0 and 9.0 and that $\Delta\Delta G_{cl}$ is nearly constant between pH 4.0 and 10 for all the F(ab)s and, therefore, human antibodies should remain stable in this pH range. Taken together, our data and empirical calculations suggest that vaginally applied human MAbs are likely to remain stable and active throughout the duration they are likely to reside in the vagina. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Mucosal surfaces are the primary entry points for many pathogens and toxins. Most antibodies synthesized by the immune system are secreted in large quantities into the fluids that bathe mucosal surfaces, and these mucosal antibodies form a first line of immunological defense against infection (Russell et al., 1999). To enhance local immune defense, monoclonal antibodies (MAbs) can be delivered topically to mucosal surfaces, including to the vagina (Cone and Whaley, 1994). However, the human vaginal environment is acidic, and this acidity might denature MAbs delivered to the vagina. Normal microflora, predominately lactobacilli, produce sufficient lactic acid to acidify vaginal secretions to pH 3.5–4.5 (Boskey et al., 2001). Vaginal pH, measured with pH electrodes, the most accurate available method, has been observed in several studies that enrolled large numbers of women (Cohen, 1969; Heinze et al., 1989; Tevi-Benissan et al., 1997). Vaginal pH was observed in a total of 340 women in these three studies. The pH in most cases was in the range 4–6. The maximum range was 3.6–8, and 6 of 340 women had a vaginal pH below 4. Therefore, in this study we investigated the pH range 3–7.

Previous studies (Matikainen, 1984; Jiskoot et al., 1990, 1991; Vermeer and Norde, 2000) have addressed the stability and biological activity of murine monoclonal antibodies as a function of the pH. Using a murine MAb against *Chlamydia* in an enzyme-linked immunoadsorbent assay (ELISA), an early investigation (Matikainen, 1984) found that the MAb retained significant binding activity after being incubated at a pH ranging from 4 to 9 for 16–18 h. However, some of the activity at pH 4–5 was attributed to non-specific binding, perhaps due to the denaturation of the antibody. In a later study (Jiskoot et al., 1990), murine MAbs against *Neisseria meningitidis* (MN12) and against Human T cell receptor (WT31) were stored for 32 days at pH 3–10, then neutralized and tested by many techniques (ELISA, flow cytometry, gel permeation chromatography, fluorescence polarization and quenching, IEF, and western blot analysis). These MAbs lost activity at extreme pH values, most likely as a result of

precipitation at low pH and fragmentation at high pH. Further investigations of the stability of murine MAbs using fluorescence spectroscopy, ultraviolet CD, birefringence, and calorimetric methods observed the effects of low pH and temperature on protein conformation (Jiskoot et al., 1991; Vermeer and Norde, 2000). Significant conformational changes were observed only when the pH was ≥ 3.5 and, at pH 3.5 only with temperatures ≥ 50 °C. The observations indicate that acidity induced expansion and increased chain mobility of the antibody, resulting in irreversible aggregation at these extremes of pH and temperature.

Although these prior studies clearly demonstrate that murine MAbs are stable down to pH ~ 3.5 , they do not indicate the pH at which human MAbs are likely to lose activity, nor the duration they are likely to remain functional, when exposed to the acidity of the human vagina. Here we report three approaches to evaluate the probable extent and rate of loss of activity of human MAbs by vaginal acidity.

We selected several MAbs representative of those that might be delivered to the vagina to prevent unwanted pregnancy and sexually transmitted infections (Cone and Whaley, 1994). First, two MAbs, a human IgM against human sperm (H6-3C4) (Isojima et al., 1987) and a human IgG₁ against gp120 of HIV (1511) (Gorny et al., 1991, 1992), were tested by ELISA for antigen binding activity as a function of pH and duration of storage. Second, to test the stability of the third antibody as measured in biological assay, a humanized MAb against glycoprotein B of HSV (Co et al., 1991; Zeitlin et al., 1998) was incubated at acidic pH and then tested for neutralizing activity using a viral neutralization assay at pH 7. Third, structure-based calculations with empirical, continuum electrostatics methods were used to calculate the pH dependence of the net charge (Matthew et al., 1985), Z , and the electrostatic component of stability (Kao et al., 2000), $\Delta\Delta G_{el}$, of a human F(ab) and, for comparison, several mouse F(ab)s (Tormo et al., 1992; He et al., 1992; Fischmann et al., 1991; Liu et al., 1994; Wien et al., 1995; Bizebard et al., 1994; Braden et al., 1994; Suh et al., 1986; Strong et al., 1991) using the structures in the Protein Data Bank of Research Collaboratory for Structural Bioinformatics.

2. Materials and methods

2.1. Antigens

Using a protocol approved by the IRB of the Johns Hopkins University, human semen was obtained by masturbation from anonymous donors and allowed to liquify for 30 min at 37 °C prior to use. Recombinant gp120 was

obtained from the NIH AIDS Research and Reference Program (Bethesda, MD).

2.2. Monoclonal antibodies

H6-3C4, a human IgM MAb against human sperm was a gift of Dr S. Isojima (Isojima et al., 1987). For this study, a volume of tissue culture supernatant containing H6-3C4 was precipitated by an equal volume of saturated ammonium sulfate, pelleted, resuspended in one-fifth volume of 150 mM NaCl and dialyzed against 150 mM NaCl. A previously described humanized anti-HSV IgG₁ MAb directed against glycoprotein B (Co et al., 1991), expressed both in mammalian cell culture and as a ‘plantibody’ in soybean cell culture (Zeitlin et al., 1998), was used in this study. A human IgG₁ MAb against gp120 of HIV (1511), produced from a human-mouse heterohybridoma (Gorny et al., 1991; Gorny et al., 1992), was obtained from the NIH AIDS Research and Reference Program.

2.3. ELISA assays

ELISA assays of antibody activity were performed as described previously (Castle, 1995). To measure anti-sperm activity, liquefied human semen was washed with Dulbecco’s PBS (DPBS; Gibco-BRL, Gaithersburg, MD), and plated into 96-well assay plates at 2×10^5 sperm per well. The wells were dried overnight at room temperature, and then treated with absolute methanol for 10 min. To measure 1511 activity, plates were coated overnight with 1 µg/ml recombinant gp120 at 4 °C. MAbs were diluted 10^{-2} in a standard buffer (20 mM PO⁻⁴, 150 mM NaCl) adjusted to pH 3.0, 3.5, 4.0, 5.0, 6.0, or 7.0, and incubated for 1 or 20 h prior to the ELISA at room temperature. At the time of assay, the MAbs were further diluted in a final dilution series of 10^{-2} , 10^{-3} , and 10^{-4} of the starting MAb concentration in the same pH buffer in which the MAb was incubated. Wells were also washed in the same pH buffer as used during storage of the antibody. Each pH treatment was done in triplicate. The fraction of activity was extrapolated from a standard dilution curve of the same MAb incubated at pH 7.0.

To test whether losses in antibody binding were due to the effects of acid on the antigen rather than the antibody, some antigen-coated plates were treated with either pH 3.0 or 7.0 buffer for 1 h prior to the assay and tested with MAbs in pH 7.0 buffer. To test whether loss of H6-3C4 activity was reversible, a sample of H6-3C4 incubated at pH 3.0 was neutralized with 1/50 volume of 1 N NaOH just prior to the assay.

2.4. HSV-neutralization assay

The CHO cell-expressed MAb in PBS (pH 7.2) and the soybean cell-expressed MAb in TRIS buffer (pH 7.6) were diluted in 5 mM citrate buffer and adjusted to pH 3.5, 4.0, 4.5, and 5.0. MAbs were incubated for 1 or 24 h under these conditions at 37 °C. Since acidic conditions are toxic for the HSV target cells, the solutions were diluted with medium (Bartels Tissue Culture Refeeding Medium; Bartels, Issaquah, WA) to neutral pH (6.5–7.5). Treated MAbs were tested in duplicate using a previously described method (Zeitlin et al., 1996). MAbs were first four-fold serially diluted in refeeding medium and then incubated with 100 TCID₅₀ HSV-2, strain G (Virotech; Rockville, MD) for 60 min at 37 °C. The antibody-virus mixture was subsequently placed on target cells (human newborn foreskin diploid fibroblast cells; Baxter) and incubated at 37 °C for 2 days, at which point viral cytopathic effect (CPE) was evaluated. CPE was rated as follows: 4 = 75–100% disruption of monolayer, 3 = 50–75% disruption of monolayer, 2 = 25–50% disruption of monolayer, 1 = 0–25% disruption of monolayer, 0 = no disruption of the monolayer. Stock MAb at 4 °C served as a positive control, and negative controls containing no MAb demonstrated maximal CPE.

2.5. Electrostatic calculations

Structure-based calculations of the pH dependence of the net charge and the stability of antibodies were performed with the empirical solvent-accessibility modified Tanford–Kirkwood (SA-TK) model following procedures described elsewhere (Matthew et al., 1985; Kao et al., 2000).

$\Delta\Delta G_{el}$ represents the free energy from interactions between charged groups. It reflects the shifts in pK_a values of ionizable groups in the protein relative to their values in model compounds in water. The success of this method in reproducing the pK_a values of surface residues is derived from the use of high dielectric constants (Matthew et al., 1985; Kao et al., 2000). Hydration effects are not included explicitly in these calculations (Matthew et al., 1985) hence these calculations are not appropriate for buried groups. Buried groups, unlike well-solvated surface residues, will experience significant pK_a shifts towards the neutral state because the charged state is not as well solvated in the protein as in water. Therefore, the change in stability versus pH in the range pH 4–7 could be slightly steeper than is shown in Fig. 3B. For the human F(ab), there are two buried histidines that might have pK_a values shifted to more acidic pH since their neutral forms will be

energetically preferred over their charged forms. The energetic cost for every unit shift in pK_a value is 1.36 kcal. Thus an upper bound for the total cost of burial of these histidines would be 5.4 kcal/mol. Even with this upper bound, the calculations indicate the F(ab) should remain stable down to at least pH 4. Moreover, although the temperature of 25 °C was used for these calculations, since the dielectric constant of water changes minimally over the temperature range 0–50 °C the magnitude of charge-charge interactions that determine the pK_a values of surface residues are not affected by temperature over the range of temperatures investigated here.

Positive free energies calculated with this method reflect repulsive interactions between charges, and are destabilizing. The intrinsic pK_a values used in the calculations were: Lys, 10.4; Arg, 12.0; His, 6.0 or 6.6; Glu, 4.5; Asp, 4.0; C-term, 3.5; N-term, 7.0; Tyr, 10.0. In the calculations the depth of burial of all ionizable residues was set to 0 Å, the ionic strength was to 0.1 M, and the temperature set to 25 °C.

3. Results

3.1. Binding activity as a function of pH

As shown in Fig. 1A, after being exposed to acidic pH for 1 h at 20 °C and then assayed by ELISA at the same pH, the MAb against human sperm (open symbols) and the MAb against gp120 (closed symbols) lost no antigen-binding activity down to pH 4.0. At pH 3.5, the most acidic pH typically present in the human vagina, HC-3C4 retained ~80% human sperm binding activity and 1511 retained ~60% gp120 binding activity. At pH 3.0, HC-3C4 retained ~60% antigen binding activity but no anti-gp120 activity was detectable for 1511.

Fig. 1B shows that, even after exposure for 20 h, there were no losses in activity down to pH 4.0. At pH 3.5, HC-3C4 retained ~70% human sperm binding activity and 1511 retained ~50% gp120 binding activity. At pH 3.0, HC-3C4 retained ~20% antigen binding activity but no anti-gp120 activity was detectable for 1511. Upon neutralization from pH 3.0 prior to performing the ELISA assay, the binding activity of H6-3C4 doubled, returning to about 40% of its original activity. Treatment of the antigen-coated plates with a pH 3.0 buffer for 1 h (the length of time the antibody is incubated in the antigen-coated plates for the ELISA assay) prior to the assays did not reduce binding of antibodies to their antigens (data not shown).

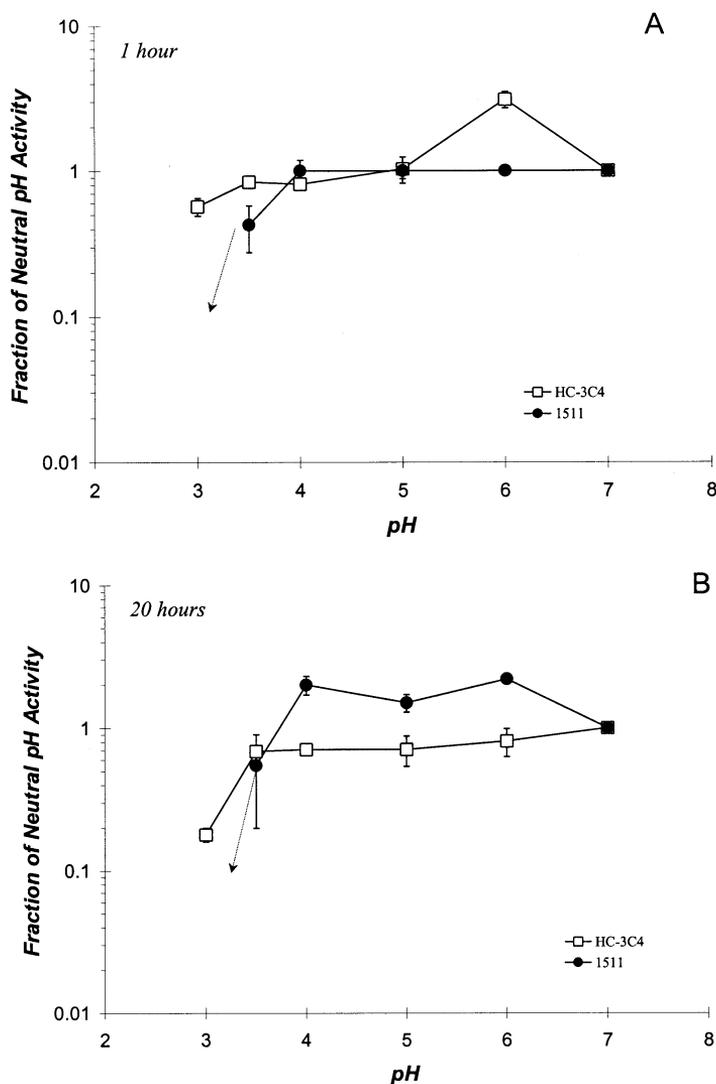


Fig. 1. Antigen-binding activity of MAbs H6-3C4 (anti-human sperm) (open box) and 1511 (anti-gp120) (filled circle) as a function of pH. MAbs were incubated for 1 h (A) or 20 h (B) at 20 °C and then tested in triplicate by ELISA in the same storage buffer. The Y-axis is a \log_{10} scale and bars represent one standard deviation of error from the mean. The arrow indicates that binding activity for 1511 was below detection at pH 3.0.

Table 1
 Identity and characteristics of the F(ab)s investigated here by computational analysis of their structures obtained from the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics

PDB Accession Codes	Antibody designation	Species	Isotype	Light chain	Antigen specificity	Reference
1bbd	8F5	Murine	IgG _{2A}	κ	Human rhinovirus	Tormo et al., 1992
<i>1dfb</i>	<i>3D6</i>	<i>Human</i>	<i>IgG₁</i>	κ	<i>Gp41 of HIV</i>	He et al., 1992
1fdl	D1.3	Murine	IgG ₁	κ	Lysozyme	Fischmann et al., 1991
1for	17-1a	Murine	IgG _{2A}	n/a	Human rhinovirus	Liu et al., 1994
1ftp	C3	Murine	IgG _{2A}	κ	Type 1 Poliovirus	Wien et al., 1995
1gig	Hc19	Murine	IgG ₁	λ	Haemagglutinin	Bizebard et al., 1994
1mlb	D44.1	Murine	IgG ₁	κ	Chicken egg-white lysozyme	Braden et al., 1994
2fbj	J539	Murine	IgA	κ	Galactan	Suh et al., 1986
6fab	36-71	Murine	IgG ₁	κ	Phenylarsonate	Strong et al., 1991

The human F(ab), 1dfb, is italicized for emphasis.

Table 2
Ionizable residues for each F(ab), The human F(ab), 1dfb, is italicized for emphasis

PDB accession codes	Acidic	Basic	His	Total	# of buried His
1bbd	40	36	6	82	2
<i>1dfb</i>	<i>37</i>	<i>40</i>	<i>5</i>	<i>82</i>	<i>2</i>
1fdl	35	37	7	79	3
1for	28	38	5	71	3
1ftp	36	35	7	78	2
1gig	31	28	8	67	3
1mlb	38	34	7	79	3
2fbj	41	35	7	83	2
6fab	39	39	4	82	2

3.2. Viral neutralization activity

Since the target cells for the HSV-2 blocking assay are acid-sensitive, this assay was performed at neutral pH and thus only the acid stability of this antibody could be evaluated, not its activity at acidic pH. Fig. 2 shows the results of testing an anti-HSV MAb produced by plant cells (open symbols) and by animal cells (closed symbols) as a function of incubation pH when incubated at 37 °C for 1 h (Fig. 2A) and for 24 h (Fig. 2B). Storage of these HSV MAbs at pH 4.0, even for 24 h (rather than 20 h used in the ELISA experiments), did not appreciably reduce their virus neutralizing activity tested at neutral pH. However, after 24 h of storage at pH 3.5, the activities of both MAbs were reduced to ~10% of their original activity.

3.3. Calculated charge and stability as a function of pH

The F(ab)s investigated here are listed in Table 1; the number of charged residues in each Fab is shown in Table 2; and the calculated net charge, Z , is shown as a function of pH in Fig. 3A. On average, these F(ab)s have nearly equal numbers of acidic and basic residues, and relatively few histidines. The human F(ab) has 37 acidic residues, 40 basic residues, and five histidines, two of which are buried. The calculated isoionic points ranged from pH 6.5 to pH 9.4. The three regions in the curves of Z versus pH correspond to the ionization of acidic residues (as pH increases from 0 to 5), ionization of histidines (pH 4.5–8.5), and ionization of lysines, arginines and tyrosines (pH 8.5 upwards). The shallow slope of these calculated titration curves between pH 5 and 9 indicates that the net charge changes relatively little over this broad pH range; this shallow slope occurs because of the low number of histidines compared with the total number of

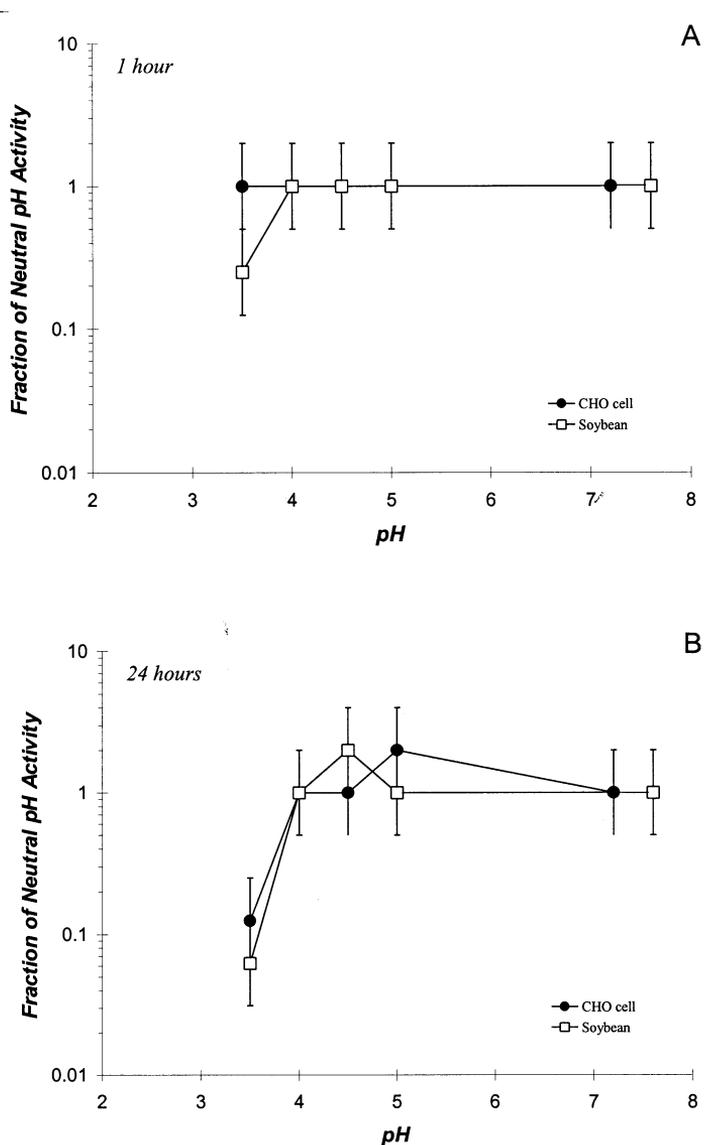


Fig. 2. Neutralizing activity of anti-glycoprotein B MAb expressed from CHO cells (filled circle) and soybean cells (open box) as function of the pH. The MAbs were incubated for 1 h (A) and 24 h (B) at 37 °C, neutralized, and tested in duplicate for activity by a HSV-2 neutralization assay. The Y-axis is a log₁₀ scale and the bars represent errors inherent in each measurement that result from assaying by two-fold serial dilutions (Zeitlin et al., 1998).

ionizable residues. Note that the titration curve of the human F(ab) was indistinguishable from those for all eight mouse F(ab)s.

3.4. Calculated pH dependence of stability

The data in Fig. 3B show the calculated pH dependence of stability of the F(ab)s. $\Delta\Delta G_{el}$ was least sensitive to pH between pH 4 and 10. Again, this broad range of stability results from the relatively low number of histidines

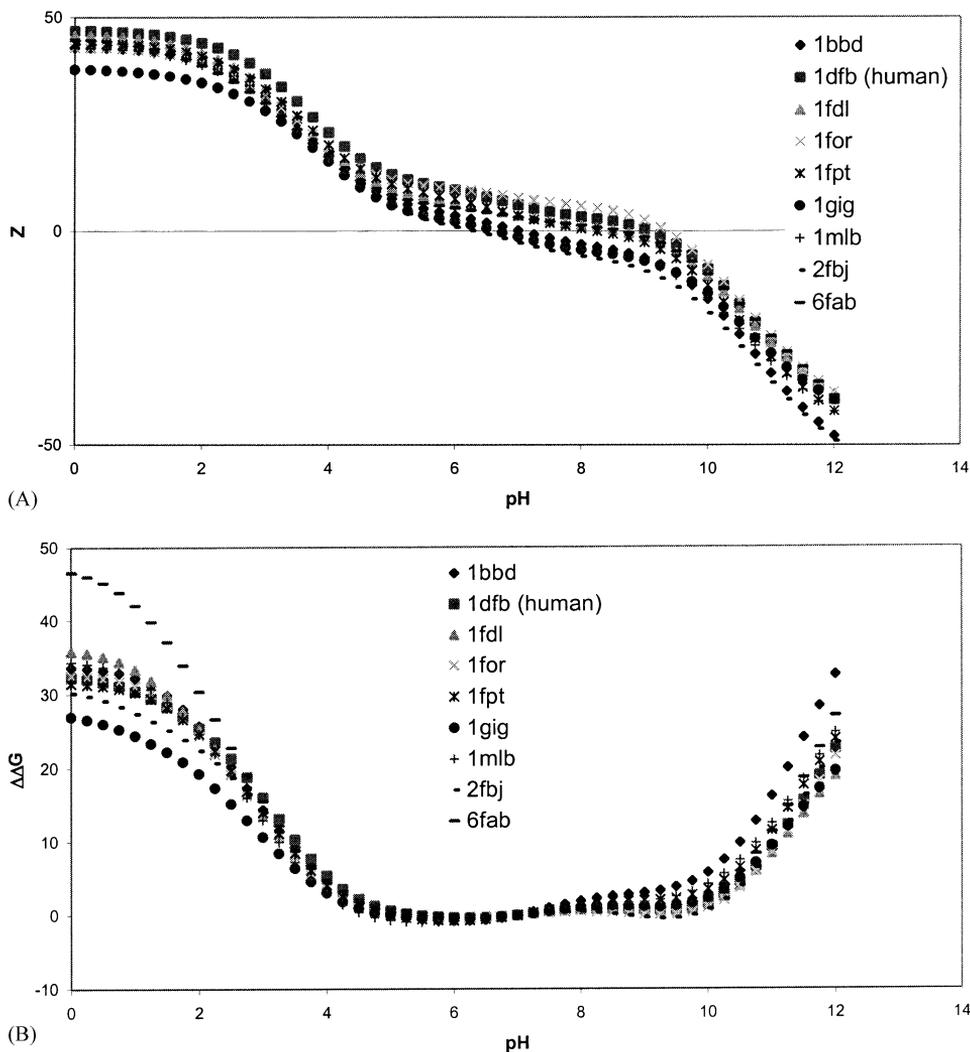


Fig. 3. (A) Calculated net charge, Z , and (B) calculated electrostatic free energy, $\Delta\Delta G_{el}$, as a function of pH for 1 human and 8 murine F(ab)s.

in F(abs). The rise in $\Delta\Delta G_{el}$ below pH 4 (and hence the decrease in stability) results from a loss in attractive interactions between basic and acidic residues as carboxylic groups become protonated. Similarly, the rise in $\Delta\Delta G_{el}$ above pH 10 results from the loss of attractive interactions between lysines and carboxylates as increasing numbers of the lysines become neutral. Again, the pH dependence of stability of the human F(ab) was indistinguishable from those for all eight mouse F(ab)s.

4. Discussion

The goal of this study was to determine the rate at which the acidity of the vagina is likely to denature human antibodies, especially human MAbs against sperm and pathogens that might be delivered vaginally for protection against unwanted pregnancy and sexually transmitted infections (Cone and Whaley, 1994). For two of the human MAbs, H6-3C4 and 1511, we were also able to assess binding activity at acidic pH by ELISA. We chose three representative human or humanized MAbs to test the effects of acidic pH on antibody activity. H6-3C4, a human IgM that binds to a sperm-surface carbohydrate epitope, was derived from an immune infertile but otherwise healthy women (Isojima et al., 1987). MAb 1511, a human IgG₁, binds to gp120 and can block HIV infection in vitro (Gorny et al., 1991, 1992). The anti-HSV-2 antibody is a recombinant humanized murine IgG₁ against glycoprotein B of HSV-2 (Co et al., 1991; Zeitlin et al., 1998).

The structure, function and amino acid sequence of immunoglobulins are conserved between species (Frazer and Capra, 1999), especially outside the hypervariable regions that form the antigen-binding sites. Since antigen binding sites are unique for each MAb and may have unique pH sensitivities, to examine the pH sensitivity of the F(ab) backbone structure we calculated the effects of pH on net charge, Z , and the electrostatic component of free energy, $\Delta\Delta G_{el}$, on one human F(ab) the only one available in the Protein Data Bank and on eight murine F(ab)s whose peptide sequences, and X-ray structures, are known. Not surprisingly, both Z and $\Delta\Delta G_{el}$ for the human F(ab) were very similar to those of the mouse F(ab)s throughout the pH range examined. On the basis of peptide sequence, structure, and charge distribution, these calculations indicate that human F(ab)s should be comparable in acid-sensitivity to the murine F(ab)s studied previously (Matikainen, 1984; Jiskoot et al., 1990, 1991; Vermeer and Norde, 2000).

As can be seen by comparing the effects of pH on antigen-binding in Fig. 1, neutralization in Fig. 2, and calculated net charge and electrostatic contribution to stability in Fig. 3, all three approaches yielded very similar

results: From pH 7 down to 4 no loss in binding or neutralization activity was detected; the calculations indicate there is only a slight increase in net positive charge and a small change in the stability. Below pH 4, binding and neutralizing activities began to decrease both as the pH decreased and as storage time increased, consistent with the loss in electrostatic stability indicated by the increase in $\Delta\Delta G_{el}$ that occurs in this pH range. However, human IgM H6-3C4 retained nearly full activity down to pH 3.5 after being incubated for 20 h at room temperature; whether this seemingly greater stability is a feature of IgMs, perhaps due to the presence of the J chain, is uncertain. Not surprisingly, antibody activity decreased significantly at pH 3.0, a pH at which antigens are commonly eluted from immunoaffinity liquid chromatographic columns. As indicated in Fig. 3, F(ab) structure is well-designed to function over a broad range of pH by virtue of having very few histidines: Over a million-fold change in proton activity the net charge Z changes only slowly, and the electrostatic stability of both murine and human F(ab)s is nearly constant. It is noteworthy that semen contains a strong alkaline buffer that neutralizes vaginal acidity (Masters and Johnson, 1966; Fox et al., 1973). Thus, binding activity at low pH may not be an absolute requirement for vaginal protection as long as the antibody is stable against the effects of acid. Even when acid induces a loss in activity, some MAbs may partially recover activity when neutralized, as demonstrated by the increase in H6-3C4 antibody activity after pH neutralization, and thus may recover activity at a time when protection is most needed.

The results presented here on human MAbs are consistent with previous extensive observations of the effect of acidic pH on murine antibodies (Matikainen, 1984; Jiskoot et al., 1990, 1991; Vermeer and Norde, 2000). We did not evaluate the effects of both temperature and acidity on antibody binding activity directly and it is plausible that temperature may accelerate losses in stability. However, the effect of pH 3.5 on a murine antibody appeared to be temperature insensitive below 50 °C (Vermeer and Norde, 2000). The similarities of murine and human antibodies demonstrated by our empirical calculations and other investigations as well as genetic conservation between the two species suggest that temperatures below 50 °C will not significantly accelerate acid-induced antibody instability. Thus, human MAbs are expected to be similarly acid-tolerant at body temperatures, with most MAbs remaining stable and active down to acidic pH in the range 3.5–4, but further explorations of the combined effects of temperature and acidity on antibody stability and activity are needed.

Only one study of vaginal residence time has been performed in humans (Moench et al., 2001): A single dose of polyclonal human IgG (Rhogam) was delivered vaginally, and, at various time intervals with different participants, a single lavage sample was obtained and assayed for antigen-binding

activity by ELISA at pH 7. Antigen-binding activity declined after vaginal delivery with a half-life of about 9 h. These *in vivo* results taken together with the *in vitro* and computational results reported here suggest that the decline in antibody titer in vaginal lavage samples obtained as a function of time after antibody delivery was most likely due to shedding of vaginal secretions or degradation by endogenous proteases rather than to acid-denaturation.

Finally, we detected no difference in the acid-stability of the HSV-2 MAb produced by mammalian cells compared with the antibody produced by plant cells. Plantibodies might be produced by large-capacity, low cost methods (Hiatt and Ma, 1993) and, therefore, be most appropriate for products that deliver monoclonal antibodies to the vagina for protection against unwanted pregnancy and sexually transmitted infections (Zeitlin et al., 2000).

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References

- Bizebard, T., Daniels, R., Kahn, R., Golinellipimpaneau, B., Skehel, J.J., Knossow, M., 1994. Refined 3-dimensional structure of the FAb fragment of a murine IgG1, lambda antibody. *Acta Crystallogr. D Biol. Crystallogr.* 50, 768.
- Boskey, E.R., Cone, R.A., Whaley, K.J., Moench, T.R., 2001. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum. Reprod.* 16, 1809–1813.
- Braden, B.C., Souchon, H., Eisele, J.L., Bentley, G.A., Bhat, T.N., Navaza, J., Poljak, R.J., 1994. Three-dimensional structures of the free and the antigen-complexed Fab from monoclonal anti-lysozyme antibody D44.1. *J. Mol. Biol.* 243, 767–781.
- Castle, P.E., 1995. Contraceptive effect of sperm-agglutinating monoclonal antibodies in rabbits. Ph.D. Thesis, Johns Hopkins University.
- Co, M.S., Deschamps, M., Whitley, R.J., Queen, C., 1991. Humanized antibodies for antiviral therapy. *Proc. Natl. Acad. Sci. USA* 88, 2869–2873.
- Cohen, L., 1969. Influence of pH on vaginal discharges. *Br. J. Vener. Dis.* 45, 241–247.
- Cone, R.A., Whaley, K.J., 1994. Monoclonal antibodies for reproductive health: Part I. preventing sexual transmission of disease and pregnancy with topically applied antibodies. *Am. J. Reprod. Immunol.* 32, 114–131.

- Fischmann, T.O., Bentley, G.A., Bhat, T.N., Boulot, G., Mariuzza, R.A., Phillips, S.E., Tello, D., Poljak, R.J., 1991. Crystallographic refinement of the three-dimensional structure of the FabD1.3-lysozyme complex at 2.5-Å resolution. *J. Biol. Chem.* 266, 12915–12920.
- Fox, C.A., Meldrum, S.J., Watson, B.W., 1973. Continuous measurement by radio-telemetry of vaginal pH during human coitus. *J. Reprod. Fertil.* 33, 69–75.
- Frazer, J.K., Capra, J.D., 1999. Immunoglobulins: structure and function. In: Paul, W.E. (Ed.), *Fundamental Immunology*. Lippincott-Raven Publishers, Philadelphia, PA, pp. 37–74.
- Gorny, M.K., Xu, J.-Y., Gianakakos, V., Karwowska, S., Williams, C., Sheppard, H.W., Hanson, C.V., Zolla-Pazner, S., 1991. Production of site-selected neutralizing human monoclonal antibodies against the third variable domain of the human immunodeficiency virus type 1 envelope glycoprotein. *Proc. Natl. Acad. Sci. USA* 88, 3238–3242.
- Gorny, M.K., Conley, A.J., Karwowska, S., Buchbinder, A., Xu, J.-Y., Emini, E.A., Koenig, S., Zolla-Pazner, S., 1992. Neutralization of diverse human immunodeficiency virus type 1 variants by an anti-V3 human monoclonal antibody. *J. Virol.* 66, 7538–7542.
- He, X.M., Ruker, F., Casale, E., Carter, D.C., 1992. Structure of a human monoclonal antibody Fab fragment against gp41 of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* 89, 7154–7158.
- Heinze, T., Riedewald, S., Saling, E., 1989. Determination of vaginal pH by pH indicator strip and by pH micro electrode. *J. Perinat. Med.* 17, 477–479.
- Hiatt, A., Ma, J.K., 1993. Characterization and applications of antibodies produced in plants. *Int. Rev. Immunol.* 10, 139–152.
- Isojima, S., Kameda, K., Tsuji, Y., Shigeta, M., Ikeda, Y., Koyama, K., 1987. Establishment and characterization of a human hybridoma secreting monoclonal antibody with high titers of sperm immobilizing and agglutinating activities against human seminal plasma. *J. Reprod. Immunol.* 10, 67–78.
- Jiskoot, W., Beuvery, E.C., de Koning, A.D.M., Herron, J.N., Crommelin, D.J.A., 1990. Analytical approaches to the study of monoclonal antibodies. *Pharm. Res.* 7, 1234–1241.
- Jiskoot, W., Bloemendal, M., van Haeringen, B., Beuvery, E.C., Herron, J.N., Crommelin, D.J.A., 1991. Non-random conformation of a mouse IgG2a monoclonal antibody at low pH. *Eur. J. Biochem.* 201, 223–232.
- Kao, Y., Fitch, C.A., Bhattacharya, S., Sarkisian, C.J., Lecomte, J.T.J., García-Moreno, E.B., 2000. Salt effects on ionization equilibria of histidines in myoglobin. *Biophys. J.* 79, 1637–1654.
- Liu, H., Smith, T.J., Lee, W.M., Mosser, A.G., Rueckert, R.R., Olson, N.H., Cheng, R.H., Baker, T.S., 1994. Structure determination of an Fab fragment that neutralizes human rhinovirus 14 and analysis of the Fab-virus complex. *J. Mol. Biol.* 240, 127–137.
- Masters, W.H., Johnson, V.E., 1966. *Human Sexual Response*. Little and Brown, Boston, MA.
- Matikainen, M.T., 1984. Effect of pH on activity of monoclonal antibodies. *J. Immunol. Methods* 75, 211–216.
- Matthew, J.B., Gurd, F.R.N., García-Moreno, E.B., Flanagan, M.A., March, K.L., Shire, S.J., 1985. pH dependent processes in proteins. *CRC Crit. Rev. Biochem.* 18, 91–197.
- Moench, T.R., Blumenthal, P., Cone, R.A., Whaley, K., 2001. Antibodies may provide prolonged microbicidal activity due to their long residence time in the vagina. *AIDS* 15, S42.
- Russell, M.W., Killian, M., Lamm, M.E., 1999. Biological activities of IgA. In: Ogra, P.L., et al. (Eds.), *Mucosal Immunology*, 2nd ed. Academic Press, New York, pp. 225–240.

- Strong, R.K., Campbell, R., Rose, D.R., Petsko, G.A., Sharon, J., Margolies, M.N., 1991. Three-dimensional structure of murine anti-*p*-azophenylarsonate Fab 36-71. 1. X-ray crystallography, site-directed mutagenesis, and modeling of the complex with hapten. *Biochemistry* 30, 3739–3748.
- Suh, S.W., Bhat, T.N., Navia, M.A., Cohen, G.H., Rao, D.N., Rudikoff, S., Davies, D.R., 1986. The galactan-binding immunoglobulin Fab J539: an X-ray diffraction study at 2.6-Å resolution. *Proteins* 1, 74–80.
- Tevi-Benissan, C., Belec, L., Levy, M., Schneider-Fauveau, V., Si Mohamed, A., Hallouin, M.C., Matta, M., Gresenguet, G., 1997. In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clin. Diagn. Lab. Immunol.* 4, 367–374.
- Tormo, J., Stadler, E., Skern, T., Auer, H., Kanzler, O., Betzel, C., Blaas, D., Fita, I., 1992. Three-dimensional structure of the Fab fragment of a neutralizing antibody to human rhinovirus serotype 2. *Protein Sci.* 1, 1154–1161.
- Vermeer, A.W.P., Norde, W., 2000. The thermal stability of immunoglobulin: Unfolding and aggregation of a multi-domain protein. *Biophys. J.* 78, 394–404.
- Wien, M.W., Filman, D.J., Stura, E.A., Guillot, S., Delpyroux, F., Crainic, R., Hogle, J.M., 1995. Structure of the complex between the Fab fragment of a neutralizing antibody for type 1 poliovirus and its viral epitope. *Nat. Struct. Biol.* 2, 232–243.
- Zeitlin, L., Whaley, K.J., Sanna, P.P., Moench, T.R., Bastidas, R., De Logu, A., Williamson, R.A., Burton, D.R., Cone, R.A., 1996. Topically applied human recombinant monoclonal IgG1 antibody and its Fab and F(ab')₂ fragments protect mice from vaginal transmission of HSV-2. *Virology* 225, 213–215.
- Zeitlin, L., Olmsted, S., Moench, T.R., Co, M.S., Martinell, B.J., Paradkar, V.M., Russell, D.R., Queen, C., Cone, R.A., Whaley, K.J., 1998. A humanized monoclonal antibody produced in transgenic plants for immunoprotection the vagina against genital herpes. *Nat. Biotech.* 16, 1361–1364.
- Zeitlin, L., Cone, R.A., Moench, T.R., Whaley, K.J., 2000. Preventing infectious disease with passive immunization. *Microb. Infect.* 2, 7001–7008.