

Development of a Kinetic Model To Describe the Effective Rate of Antibody Oxidation by Periodate

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The oxidation of antibody carbohydrate residues by periodate is a common approach used for site-specific antibody modification and immobilization. This study sought to develop a general kinetic model that could be used to describe the effective rate of this oxidation for process control. A detailed analysis of previous data collected for rabbit immunoglobulin G in the presence of excess periodate indicated that the reaction followed a pseudo-first-order mechanism in which two general classes of sites were being oxidized. The first class of sites was oxidized fairly rapidly (i.e., within 15–30 min), while the second class of sites reacted over the course of several hours. From these results, an equation was developed that gave a good fit under a variety of reaction conditions to the production of oxidized sites available for coupling with a hydrazide label. On the basis of this equation, data obtained at several periodate concentrations under the same pH and temperature conditions were used to estimate the apparent rate and equilibrium constants for the oxidation of each class of sites. The values obtained by using this approach could be used not only to predict the effective rate of oxidation at other periodate concentrations but also to provide information on the individual steps involved in the oxidation process.

INTRODUCTION

The oxidation of antibodies by periodate is a popular means for the site-specific modification of antibodies for coupling to solid supports or labeling agents. A number of reviews on the applications of periodate-treated antibodies have appeared previously in the literature (e.g., refs 1 and 2); recent examples of specific applications include those discussed in refs 4–7. During the treatment of antibodies with periodate, diol groups located in the carbohydrate chains of an antibody are cleaved to form aldehyde groups. These aldehydes can then be used as sites for attachment of the antibody to agents that contain free amine or hydrazide residues. The main advantage of this approach is that the carbohydrate residues on antibodies are usually located in regions that are distant from the antigen binding sites. Thus, the use of these residues for antibody modification should result in little or no change in the antibody's final binding activity (1, 2).

Despite the potential usefulness of antibody treatment by periodate, only limited quantitative information is available regarding the actual nature of this reaction. It has been shown that the periodate oxidation of glycoproteins depends on such factors as periodate concentration, reaction time, pH, and temperature (3–6, 8, 9). However, many previous studies with antibodies have examined only some of these parameters in any detail. To overcome this limitation, our group has collected data on the oxidation of rabbit immunoglobulin G (rabbit IgG) under a variety of reaction conditions (10). From this work, a set of empirical guidelines was developed for obtaining various levels of antibody oxidation while also minimizing the loss of antibody activity that occurs as a result of the oxidation process. However, a general model is still

needed that can be used along with this information to predict and follow the periodate treatment of antibodies to obtain a precise means of controlling or adjusting this reaction.

The goal of the present study is to use previous data obtained in work with rabbit IgG to develop a kinetic model and associated equations that can be used to describe the effective degree of antibody oxidation (i.e., the production of aldehyde groups that are available for reaction with a hydrazide- or amine-containing reagent) as a function of time and various reaction conditions that are commonly used during antibody treatment with periodate. This type of model would be potentially useful in allowing for improved control and selection of those conditions that are needed to obtain a given degree of antibody modification for labeling or immobilization. The availability of such a model would also be valuable as a means for studying the mechanisms involved in the periodate oxidation of antibodies or other glycoproteins.

MATERIALS AND METHODS

As discussed previously (10), polyclonal rabbit IgG was selected as the model antibody preparation for this work since it is a common immunological reagent and a number of previous studies on antibody oxidation by periodate have been based, at least in part, on rabbit IgG (5, 8). Furthermore, it was felt that the use of a polyclonal system would help to avoid the variability that has been noted in previous studies examining the effects of periodate oxidation on monoclonal antibodies (3–5, 9). Also, since polyclonal antibody preparations are composed of a large number of distinct antibody lines, this type of system should be fairly representative of the average behavior that would be expected for individual antibody populations when they are treated by a reagent such as periodate.

The polyclonal rabbit IgG was oxidized with periodate as described in ref 10. The effective number of aldehyde groups that were available for coupling was determined by labeling with the hydrazide-containing dye Lucifer yellow CH (LyCH), also as described previously (10).

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LyCH was obtained from Aldrich (Milwaukee, WI); rabbit IgG and the *p*-periodic acid reagent used to make the periodate solutions (see ref 10 for further details) were from Sigma Chemical Co. (St. Louis, MO). Other chemicals used in this study were of reagent grade quality or better. The degree of antibody labeling by LyCH that was obtained under each set of oxidation conditions was measured by flow injection analysis (FIA), as performed according to procedures given in ref 11.

The simplex optimization method used for analysis of the kinetics of antibody oxidation was performed by a program written in Microsoft QuickBASIC (Redmond, WA). This program was based on a modified version of the procedure given by Noggle (12). For simplex optimization performed with the two-site model, the variance of the fit was calculated by dividing the sum of the squares of the residuals by $N - 4$ degrees of freedom, where N is the total number of points present in the original data set. All simplex optimization studies were carried out by examining the results obtained with at least eight different combinations of estimates for the initial parameters, as selected from points scattered throughout the range of allowable results. All fits were evaluated on the basis of both the residuals or variances that were obtained and a visual comparison of the predicted response versus the experimental data.

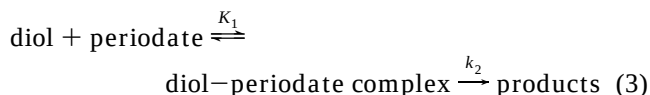
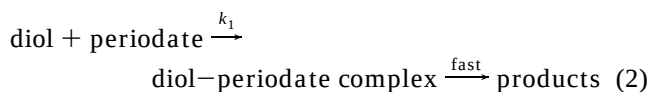
RESULTS AND DISCUSSION

To obtain a model that described the effective rate of antibody oxidation by periodate, an analysis was performed on data previously collected for rabbit IgG in the presence of a known excess of periodate and at various pH values and temperatures (10). The range of test conditions included those typically employed in previous studies involving the periodate oxidation of antibodies (1–9) or other glycoproteins (13–16). In each case, periodate was initially present in at least a 150-fold molar excess versus the amount of antibody in the sample; this resulted in a pattern that consisted of a rapid increase in oxidized sites at short reaction times (i.e., <15–30 min) followed by a much slower increase over longer reaction times (10). The same type of pattern has been noted by others not only in the periodate oxidation of antibodies but also in the treatment of additional glycoproteins by periodate (5, 6, 11, 15–17).

As a first step in developing a kinetic model for antibody oxidation, consideration was given to the various mechanisms that have been proposed for the periodate treatment of other, low molecular weight compounds. For example, studies with various small 1,2-diol compounds have found that periodate oxidation can generally be described by a two-step process:



The first step of this reaction involves the reversible formation of a cyclic intermediate complex between the diol group and 1 equiv of periodate. After this complex is formed, the diol is cleaved at its carbon-carbon bond to form two aldehyde groups (18). There are two extreme cases that can arise from the model in eq 1. In the first case, formation of the diol-periodate complex is the slow step, while in the second case the formation of products from the cyclic complex is rate-limiting. These two situations are represented by the reaction schemes shown in eqs 2 and 3, respectively.



In this series of reactions, k_1 is the forward rate constant for complex formation, k_2 is the rate constant for product formation, and K_1 is the association equilibrium constant for the diol-periodate complex. Both cases in eqs 2 and 3 have been observed in experimental systems (18). For example, simple diols such as ethylene glycol and 2,3-butyleneglycol readily form a cyclic intermediate with periodate but have slow cleavage reactions. On the other hand, highly substituted or hindered diols can have slow formation of the cyclic intermediate, followed by more rapid cleavage of the diol carbon-carbon bond (18).

When a large excess of periodate is present in solution, as was true for rabbit IgG in this study, it is possible to represent either of the cases in eqs 2 or 3 by a pseudo-first-order rate expression. The resulting integrated equations, expressed in terms of the formation of product, are given below (19):

$$B_t = nA_0(1 - e^{-k_{app}t}) \quad (4)$$

or

$$\ln[1 - (B_t/nA_0)] = -k_{app}t \quad (5)$$

In eqs 4 and 5, B_t represents the amount of product that is present at time t , A_0 is the initial amount of the reactant, k_{app} is the apparent rate constant for the conversion of A to B, n represents the moles of B that are produced per mole of A that is consumed, and nA_0 is the maximum amount of product (i.e., labeling sites in this study) that can result under the given reaction conditions. Note that the amount of B and A in eqs 4 and 5 can be expressed in terms of either concentrations, moles, or mole ratios (e.g., moles of LyCH per mole of IgG) as long as both A and B are present in the same solution or reaction compartment. Furthermore, the use of the proportionality constant n in these equations is useful in that it allows the reaction rate to be expressed in terms of any product B whose production is proportional with, but not necessarily identical to, the consumption of A (i.e., where A represents diol groups in this case). This makes eqs 4 and 5 valuable in describing antibody oxidation in terms of the effective number of labeling sites that are produced per antibody, since either one or two aldehyde groups can be generated per oxidation event and not all of these aldehydes might be available for labeling due to steric hindrance or other constraints (see discussion at the end of this section).

Although either of the systems in eq 2 or 3 would be expected to fit a pseudo-first-order model in the presence of excess periodate, the actual meaning of k_{app} will differ depending on which situation is present. In the case when complex formation is rate-limiting (eq 2), the value A_0 is equal to the initial amount of diol groups that can take place in the oxidation reaction and the value of k_{app} is given by the equation

$$k_{app} = k_1[\text{periodate}] \quad (6)$$

where [periodate] is the total concentration of periodate present in solution and k_1 is the forward rate constant for complex formation, as defined previously. In the

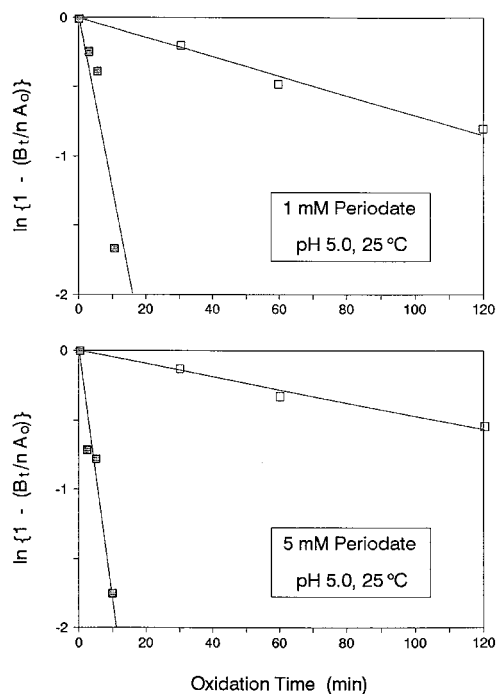


Figure 1. Fit of a first-order rate expression to data for the initial reaction of rabbit IgG with periodate (■) and over longer reaction times after correcting the net response for the contribution predicted by the initial rate analysis (□). This correction assumes the net rate of oxidation can be described by parallel reactions with different reacting sites but similar products (19).

situation when cleavage of the carbon–carbon bond is rate-limiting (eq 3), A_0 is still equal to the total amount of diol groups (both free and complexed) that is present prior to the cleavage reaction; however, k_{app} is now given by the expression

$$k_{app} = k_2 (K_1[\text{periodate}]) / (1 + K_1[\text{periodate}]) \quad (7)$$

The fact that these two cases result in different equations for k_{app} will be used later to help determine which mechanism best describes the oxidation of antibodies by periodate.

Early work in this study examined the fit of a single first-order expression to data obtained for the oxidation of rabbit IgG at pH 5.0 and 25 °C between 0 and 240 min. When this was done, large deviations were noted between the data and the expected response at all of the periodate concentrations that were tested (i.e., 1–100 mM). However, data obtained at short reaction times (0–10 min) did appear to have reasonable agreement with the pseudo-first-order model. This is shown in Figure 1, which illustrates the fit of eq 5 to data obtained at periodate concentrations of 1 and 5 mM. Similar results were obtained when using up to 10 mM periodate. The correlation coefficients for these plots ranged from –0.9459 to –0.9783 for the four to five points in each graph between 0 and 15 min. This indicated that even though data measured over long times did not follow the pseudo-first-order model, the initial rate did seem to fit this type of response. The best-fit values for k_{app} that were obtained from the slopes of these plots were between 0.2 and 0.3 min^{-1} [i.e., $(3\text{--}5) \times 10^{-3} \text{ s}^{-1}$]. The estimated values for nA_0 , as determined by iterative testing, varied from 0.3 to 1.5 mol of LyCH/mol of IgG.

By assuming that the pseudo-first-order fit to the initial data represented a single class of fast-reacting sites, it was possible to use this fit to estimate how many labeling sites were produced by this oxidation process at

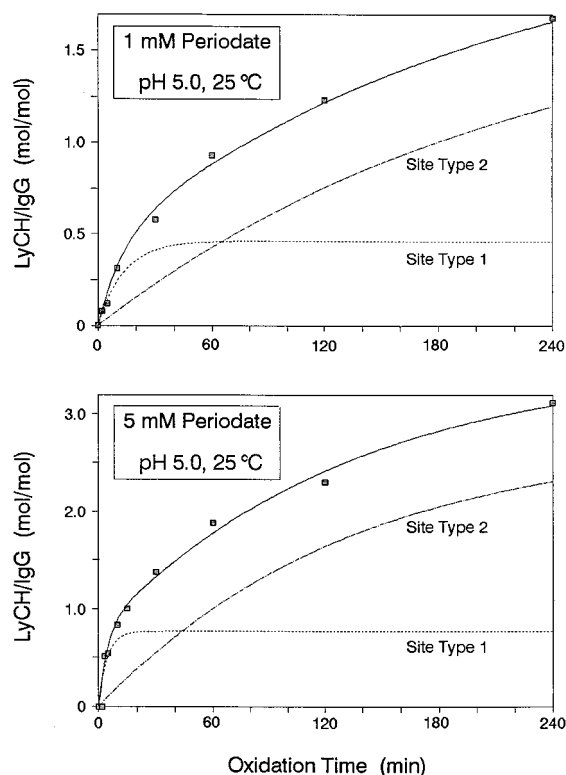


Figure 2. Overall fit of oxidation data for rabbit IgG (■) to a model in which two separate classes of sites for oxidation are present (types 1 and 2). The lines shown in this graph were calculated using eqs 4 and 6, along with the best-fit parameters obtained through simplex optimization.

any given reaction time. These values were then subtracted from the total number of labeling sites that were actually measured. After this was done, the remaining LyCH/IgG levels were replotted according to eq 5, as shown in Figure 1. On the basis of this approach, it was found that the remaining oxidation data again showed good agreement with a pseudo-first-order model. The fits obtained for periodate concentrations of 1–10 mM and reaction times of 0–240 min had correlation coefficients of –0.9641 to –0.9989 over four to five data points. The best-fit values measured for k_{app} were between 0.004 and 0.007 min^{-1} [i.e., $(7\text{--}12) \times 10^{-5} \text{ s}^{-1}$], and the estimated values for nA_0 ranged from 1.7 to 4 mol of LyCH/mol of IgG.

From the results in Figure 1, a model was proposed for the antibody oxidation process in which two distinct classes of sites were present, each following pseudo-first-order kinetics in the presence of excess periodate. On the basis of this model and eq 4, the following equation was developed to describe the overall oxidation process

$$B_t = (nA_0)_1 [1 - e^{-k_{app,1}t}] + (nA_0)_2 [1 - e^{-k_{app,2}t}] \quad (8)$$

where the subscripts “1” and “2” refer to the parameters for the first and second types of sites, respectively.

The fit of eq 8 to each set of oxidation data was tested by using simplex optimization to obtain the best-fit values for $(nA_0)_1$, $(nA_0)_2$, $k_{app,1}$, and $k_{app,2}$. Figure 2 shows the fits that were obtained for data measured in presence of 1–5 mM periodate at 5.0 and 25 °C. In both of the given examples, excellent agreement was seen between the two-site model and the experimental results over the entire range of reaction times that were sampled. The same type of fit was noted when using other periodate concentrations up to 10 mM. The variance of these fits was between 0.003 and 0.005 over 8–10 data points. The

Table 1. Estimated Equilibrium and Rate Constants for the Periodate Oxidation of Rabbit IgG at pH 5.0 and 25 °C^a

oxidation site	nA_0 (mol of LyCH/ mol of IgG)	K_1 (M ⁻¹)	k_2 (s ⁻¹)
site class 1	0.7 (± 0.1)	240 (± 90)	$8 (\pm 3) \times 10^{-3}$
site class 2	1.9 (± 0.7)	600 (± 400)	$3 (\pm 1) \times 10^{-4}$

^a The values in parentheses represent a range of ± 1 SD. The value of nA_0 , or the effective moles of labeling sites that could be generated per mole of IgG, represents the mean result obtained by simplex optimization over five periodate concentrations between 1 and 10 mM and using two separate batches of antibody.

best-fit values for $k_{app,1}$ and $k_{app,2}$ in these plots ranged from 1×10^{-3} to 8×10^{-3} s⁻¹ and from 7×10^{-5} to 40×10^{-5} s⁻¹, respectively, with both of these parameters increasing with periodate concentration. The size of $(nA_0)_1$ and $(nA_0)_2$ varied between 0.5–0.8 and 1.2–2.8 mol of LyCH/mol of IgG (see Table 1 for mean values) and showed only random variations as the periodate concentration was changed. Overall, these best-fit parameters gave good agreement with the k_{app} and nA_0 values that were estimated earlier by linear regression analysis, such as performed in Figure 1.

By using the best-fit parameters in Table 1 along with eq 4, it was possible to determine the contribution of each site to the overall extent of oxidation that was measured by the LyCH label. The results are included in Figure 2. The picture that results from this is one in which the first class of sites (type 1) is oxidized within 15–30 min, followed by slower oxidation of the second class of sites (type 2) over a period of at least several hours. This model is consistent with the biphasic behavior that has often been observed for the oxidation of antibodies by periodate (5, 6, 10, 11). In addition, these results agree with past work performed with another glycoprotein, orosomucoid, in which the presence of different classes of sites has been proposed as an explanation for the biphasic nature of the periodate oxidation process (20).

The antibody oxidation process was examined in more detail by studying the concentration dependence of the measured k_{app} values. This was of interest since knowing the expected relationship between k_{app} and periodate concentration would allow the effective rate of antibody oxidation to be estimated at new periodate levels once it has been established for several other periodate concentrations. To study this, plots were made of $1/k_{app}$ versus $1/[periodate]$. According to eqs 6 and 7, one of the following linear relationships should result for this type of plot if the oxidation mechanism is limited exclusively either by the rate of complex or product formation:

$$1/k_{app} = 1/(k_1[periodate]) \quad (9)$$

$$1/k_{app} = 1/(k_2K_1[periodate]) + 1/k_2 \quad (10)$$

For a reaction that is limited by the rate of complex formation, eq 9 shows that a plot of $1/k_{app}$ versus $1/[periodate]$ should result in a linear relationship with a slope of $1/k_1$ and an intercept of zero. For a reaction that is limited by the rate of breaking the diol carbon–carbon bond, eq 10 predicts that a linear response will also result but with a slope that is now given by $1/(k_2K_1)$ and a nonzero intercept that is equal to $1/k_2$.

Figure 3 shows the graph that was generated when a plot of $1/k_{app}$ for site type 1 was made versus $1/[periodate]$. A linear relationship was obtained, as predicted by eqs 9 and 10, with a correlation coefficient of 0.9740 over five points. The best-fit slope for this graph was determined

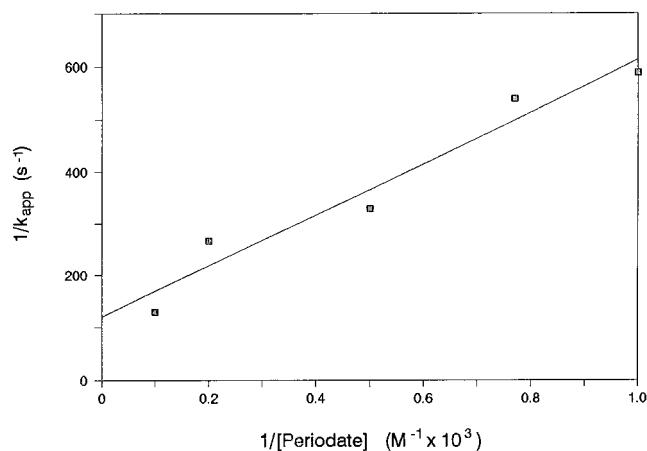


Figure 3. Change in k_{app} with periodate concentration at site type 1 on rabbit IgG. The best-fit parameters are given in the text. The results shown were obtained at pH 5.0 and 25 °C using periodate concentrations of 1, 1.3, 2, 5, and 10 mM.

to be 0.49 ± 0.07 M s (1 SD), and the intercept was 120 ± 40 s. A similar plot was found for site type 2, but with more scatter being present about the best-fit line. In the latter case, a correlation coefficient of 0.7818 was obtained over five points, with a slope of 7 ± 3 M s and an intercept of 3800 ± 1900 s. These linear relationships indicate that such plots should make it easy to estimate k_{app} at new periodate concentrations once this apparent rate constant has been determined at a few surrounding periodate levels.

A further examination of plots such as those in Figure 3 helped to provide some insight into the nature of the antibody–periodate reaction. For example, the data for both classes of sites gave intercepts for plots of $1/k_{app}$ versus $1/[periodate]$ that were significantly different from zero at the 90% confidence level. According to eqs 9 and 10, this supports a model in which periodate and diol groups on the antibody rapidly formed a cyclic complex (as has been shown to form between periodate and low-mass diol compounds—see ref 18), followed by the slow cleavage of this complex to form the aldehyde products. The same mechanism has been used by Nevell (21) to describe the oxidation of cellulose by periodate. By assuming that a cleavage-limited mechanism was indeed present, it was possible to use eq 10 to determine the values of k_2 and K_1 for the two types of sites under the given pH and temperature conditions. The results are summarized in Table 1. For the proposed model, it was found that the two classes of sites had similar, weak equilibrium constants for complex formation, with both K_1 values falling within the range of 200–600 M⁻¹. However, the two classes of sites did differ in the maximum number of available aldehydes that could be generated (nA_0) and in the rate constants for diol group cleavage (k_2). In this case, site type 1 had a smaller value for nA_0 , but the value of k_2 for this type of site was about 25-fold larger than it was for the second class of oxidation sites.

The two-site model that was used in this work to describe antibody oxidation was found to work well not only at pH 5.0 and 25 °C but also at other pH and temperature conditions. Some typical examples are shown in Figure 4. The range of conditions that were examined for antibody oxidation included a pH range of 3–7 (at 10 mM periodate and 25 °C), a temperature range of 4–37 °C (at 10 mM and pH 5.0), and a concentration range of 1–100 mM periodate (at pH 5.0 and 25 °C). In all but two of these cases there was good agreement between the actual data and the two-site model, with total variances that ranged from 0.002 to 0.07 (mean, $0.02 \pm$

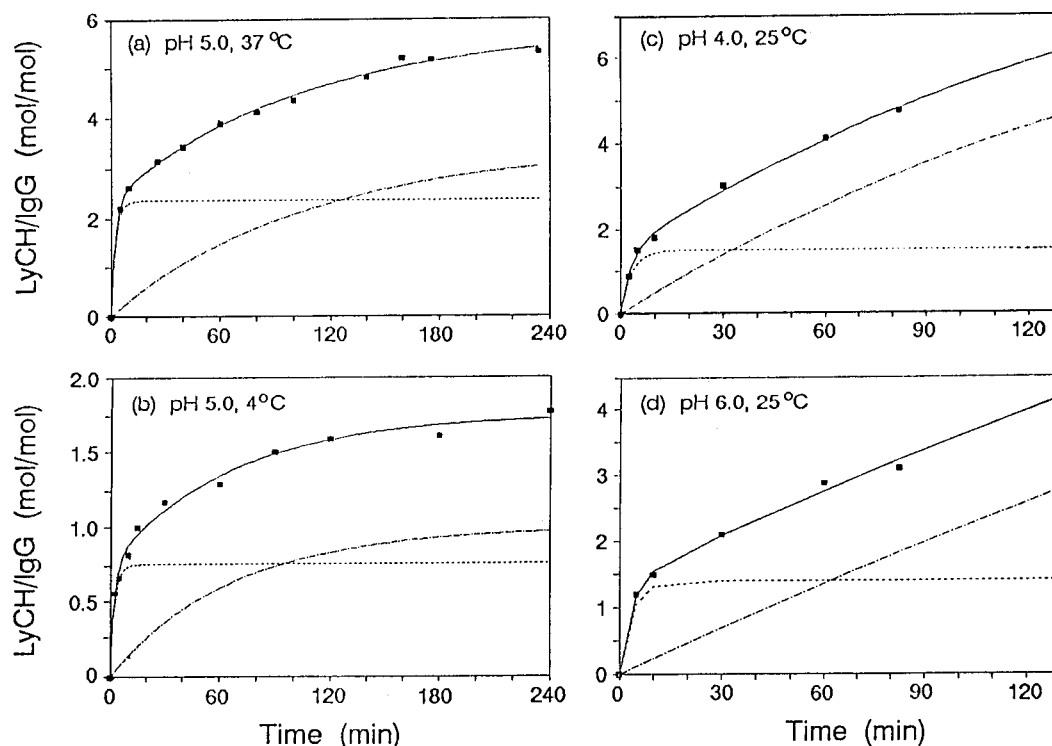


Figure 4. Fit of a two-site model to oxidation data obtained for rabbit IgG (■) under a variety of pH and temperature conditions. The dashed and dotted lines show the estimated contributions due to site types 1 and 2.

Table 2. Actual and Predicted Degrees of Antibody Oxidation by Periodate

oxidation conditions	degree of oxidation (mol of LyCH/mol of IgG) ^a	
	predicted	actual
10 min, pH 7.0, 10 mM periodate, 25 °C	1.0 (± 0.1)	0.9 (± 0.1)
30 min, pH 5.0, 10 mM periodate, 25 °C	2.1 (± 0.1)	2.0 (± 0.1)
60 min, pH 5.0, 10 mM periodate, 25 °C	2.7 (± 0.1)	2.9 (± 0.1)
60 min, pH 4.0, 10 mM periodate, 25 °C	4.1 (± 0.2)	3.6 (± 0.1)

^a The numbers in parentheses represent a range of ± 1 SD.

0.02) over 6–13 points. The only exceptions were those data sets that were measured at 50 and 100 mM periodate. In these two cases, significant deviations were seen from the two-site model at long reaction times (i.e., >1–2 h). This was expected since these same conditions were found to result in the formation of a significant amount of visible precipitate, indicating the possible presence of side reactions due to overoxidation or antibody cross-linking (10).

The accuracy and potential utility of the two-site kinetic model were next tested by using this model to predict the effective rate of periodate oxidation for rabbit IgG. This was done by first using the kinetic fits in Figures 2–4 and related graphs to choose oxidation conditions that would be expected to produce approximately one, two, three, or four active aldehyde groups per IgG. These conditions were next used to oxidize some fresh samples of normal rabbit IgG, with the final extent of oxidation then being measured and compared to the predicted level. The results are summarized in Table 2, with all four test conditions giving good agreement between the actual and predicted degrees of antibody oxidation. Each set of experimental and predicted oxidation levels overlapped within a range of 2 standard deviations, and the absolute differences in these values

varied by only –12.2% to +7.4% (mean, –4.9%). This degree of accuracy was considered to be adequate for most applications since such differences are less than or comparable to the within-day precision of 5% and day-to-day precision of 10–20% expected for the periodate oxidation of rabbit IgG under constant reaction conditions (10).

The carbohydrate structure of a typical antibody (Figure 5) reveals some clues regarding what residues on the antibody carbohydrate regions might be represented by the two types of oxidation sites in the proposed kinetic model. In rabbits and other mammals the oligosaccharides that are attached to IgG are highly heterogeneous in nature (22), with Figure 5 showing the maximum number of sugar units that can be present in a single carbohydrate chain. However, in reality many of the actual carbohydrate chains on rabbit IgG may lack the 1' fucose and/or 4'' *N*-acetylglucosamine residues, or they may contain only a partial extension of the sequences shown in the 4–7 and 4'–7' chains (22).

Although the carbohydrate regions for rabbit IgG are heterogeneous, the aldehydes that can be generated by periodate in these regions could occur through the oxidation of two different types of diol groups: (1) those present as exocyclic diols on sialic acid residues or (2) those within the cyclic structures of other sugars in the carbohydrate chain (13, 15). Of these two types of diols, those present on sialic acids have long been known to have the greatest susceptibility to oxidation by periodate (23). Sialic acids contain diol groups at both the C7–C8 and C8–C9 exocyclic regions. The oxidation of either diol group results in the formation of a free aldehyde in solution and a single aldehyde that is attached to the carbohydrate chain (i.e., $n = 1$ in the term nA_0 , assuming 100% labeling efficiency by LyCH). Studies with other glycoproteins have shown that terminal sialic acids are rapidly oxidized by periodate over a time scale matching that seen for site type 1 in this study (16, 17, 20, 24). In addition, data obtained from previous studies that have examined the extent of rabbit IgG sialylation (25) give

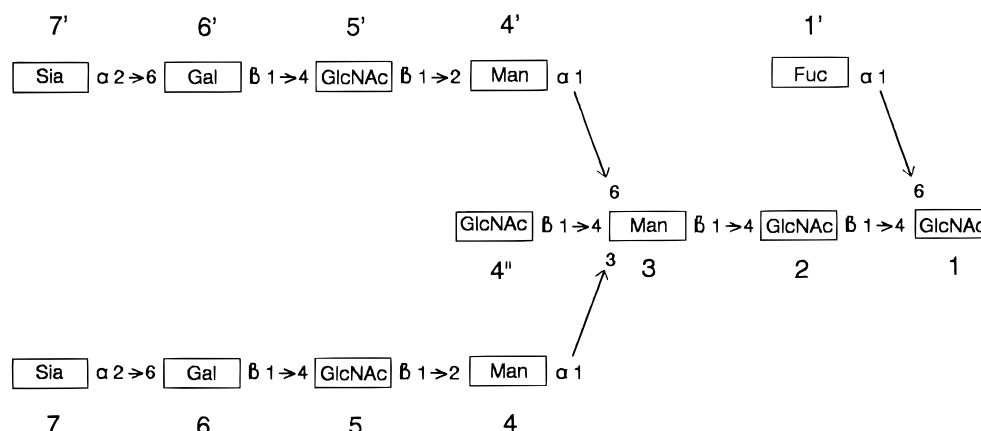


Figure 5. Sequence of the fully extended form of an *N*-linked carbohydrate region on IgG. The abbreviations indicate the locations of sialic acid (Sia), β -D-galactose (Gal), β -D-*N*-acetylglucosamine (GlcNAc), β -D-mannose (Man), and β -L-fucose (Fuc). The numbers below each residue indicate the sugar's position in the chain, based on the scheme of Rademacher et al. (22).

an average number of sialic acids per IgG that shows good agreement with the value of $(nA_0)_1$ in Table 1. On the basis of this information, it was concluded that the first class of oxidation sites (type 1) probably represents the exocyclic diol groups on the sialic acid residues.

The second general type of diol groups (i.e., those suspected to represent cyclic sugar residues) could occur at a variety of locations within the carbohydrate chains. For the fully extended structure shown in Figure 5, diol groups occur within the 1' fucose, 4'' *N*-acetylglucosamine, and two 4/4' mannose residues. Although some of these sugars have more than one pair of adjacent diol groups, a maximum of two aldehydes is produced by the reaction of periodate at any one of these residues. For carbohydrate chains with a partially extended structure, additional pairs of aldehyde groups may be produced by oxidation at a terminal galactose (at the 6- or 6'-positions in the absence of 7 or 7' sialic acids) or a terminal *N*-acetylglucosamine (at the 5- or 5'-positions in the absence of the 6–7 or 6'–7' residues). On the basis of the different combinations of sequences that have been noted in mammalian IgG (22), there is the potential to generate 8–12 aldehydes per carbohydrate chain in the absence of any sialic acid and 6–10 aldehydes per chain when both terminal sialic acids are present (i.e., 4–8 aldehydes from cyclic diols and 2 from sialic acids). Although not all of these sites may be available for labeling due to steric hindrance or other factors, the relatively small nA_0 value noted in Table 1 for site type 2 suggests that only some cyclic diol groups are represented by the second class of sites under normal oxidation conditions. Fucose is one likely source of these groups since studies with other glycoproteins have found this sugar to be readily oxidized by periodate on an hour time scale (16, 17, 20). Terminal galactose residues have also been noted to undergo relatively fast oxidation by periodate, making this sugar another candidate for the type 2 oxidation sites.

Although only a few types of residues might be represented by site type 2 under standard oxidation conditions, more residues might be included in this class of sites when harsher reaction conditions are used. For example, at an oxidation pH <4.5 or at a reaction temperature >25 °C there was an apparent increase in the value of $(nA_0)_2$ that was obtained by simplex optimization. The extent of this increase ranged from a value for $(nA_0)_2$ of 1.9 mol of LyCH/mol of IgG at pH 5.0 and 25 °C to levels of 3.4–8.8 mol of LyCH/mol of IgG at pH 5.0/37 °C and pH 3.0/25 °C, respectively. As discussed earlier, this suggests that other cyclic diols can also fit

within the two-site model but that not all of these are amenable to periodate oxidation unless suitably strong reaction conditions are used. The fact that a relatively good fit is obtained to a two-site model even under this fairly wide range of conditions indicates that if multiple residues are represented by site type 2, then the apparent rate constants for their oxidation must be fairly similar. This is useful since it avoids the need for more complex models to describe the net rate of the oxidation process under these other reaction conditions.

CONCLUSIONS

In summary, this work examined the development of a kinetic model to help describe the effective rate of oxidation of rabbit IgG by periodate to form aldehyde groups for labeling or immobilization. The model that was developed was based on a pseudo-first-order mechanism in which two distinct classes of sites were being oxidized. The first type of site was oxidized fairly rapidly (i.e., within 15–30 min), while the second type of site reacted over the course of several hours. It was proposed that these two classes of sites represented exocyclic and cyclic diol groups, respectively. From the concentration dependence of the apparent rate constants, it was further suggested that this oxidation occurred through a mechanism in which the periodate and carbohydrate residues rapidly form a reversible complex that then undergoes a slower cleavage reaction. On the basis of comparisons that have been made between the carbohydrate regions of mammalian IgG antibodies (22), it is anticipated that the same kinetic model as developed here for rabbit IgG could be applied to other species, including human, sheep, mouse, and bovine IgG.

The model and equations developed in this study are of potential fundamental importance because they provide a basis for examining the kinetic processes that take place during the oxidation of antibodies or other glycoproteins by periodate. This model and these equations are also of interest from an applied viewpoint since they could be used to describe or predict the average number of labeling or immobilization sites that will be generated on oxidized antibodies. As shown in this work, the model developed in this study appears to work under a variety of pH and temperature conditions. It also appears to have the ability to separate the overall oxidation process into the contributions of two distinct classes of sites. This latter ability is of interest since it could allow the oxidation reaction to be tailored to emphasize the treatment of a particular group of carbohydrate residues. This is already done to some extent with glycoproteins through

the use of low temperatures and neutral pH values for the oxidation of sialic acid in the presence of other sugars (14, 25–27). On the basis of the model developed in this work, it should be possible to further refine the conditions that are required for this type of selective oxidation.

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