

Calculation of Antibody Affinity in Homogeneous and Heterogeneous Systems

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Antibody affinity is an important determinant of all antibody-antigen reactions. A new computer program, AFCRV, was developed to calculate binding constants with data from a radioimmunoassay on most microcomputers in the laboratory by using constant-ratio dilution curves. Evaluation of a homogeneous or heterogeneous antibody in the presence of a single antigen can be accomplished.

Antibody affinity is defined as the attractive force or strength between an antigenic determinant and the antibody combining site (1). The strength of the reaction is dependent on numerous factors, which include the fit of the antigen with its combining site, the distance between the two reactants, the concentration of the antibody, the density of the antigen determinant, and the presence of a multivalent antibody and complex antigens. The affinity constant for an antibody-antigen reaction has been described as probably the most important overall parameter of the reaction, since it determines the sensitivity of detection of the reaction (11).

Antibody affinity has been shown to be an important determinant of the efficacy of several laboratory tests, such as hemagglutination, complement fixation, enzyme-linked immunoabsorbent assay, precipitation, and radioimmunoassay (8, 10, 12, 15). Affinity has also been implicated as an important factor in the biological effects noted in humans and animals (3, 10, 15) and should be included as an essential ingredient in the evaluation of any vaccine (15).

Antibody affinity has traditionally been measured in the laboratory by equilibrium dialysis (1). The data are analyzed by linear transform by either the Scatchard (13) or the Sips (14) equations to produce a plotted line with a specific degree of slope. Several investigators have reported that these calculations are cumbersome and inaccurate and tend to fail to linearize the curves (2, 4, 6, 16).

Nelson and Griswold (9), using a radioimmunoassay with bovine serum albumin, have presented an improved approach to the measurement of antibody affinity by reporting the concept of constant-ratio dilution (CRD) curves. In traditional dilution experiments, in which a series of dilutions of the antibody is compared with a single dilution of the antigen, a wide variation in the antigen-to-antibody ratio is measured throughout the dilution series. In CRD experiments, the antigen-to-antibody ratio is kept constant by proportional variation of both the antigen and antibody concentrations. Calculations of CRD curves is accomplished by regression analysis to produce the resultant curve of responses.

The purpose of this paper is to report the development of a new computer software program AFCRV, that calculates antibody affinity with radioimmunoassay data and a simple microcomputer in the laboratory by developing CRD curves for the desired antigen-antibody complex.

MATERIALS AND METHODS

Computer. The AFCRV computer program was written and compiled to dramatically increase the speed of the

calculations. It will run on any IBM (International Business Machines Corp., Boca Raton, Fla.) personal computer with the disk operating system and requires no special computer hardware. If the computer has an 8087 mathematical coprocessor to speed up the mathematical processing, the time required to complete an analysis of a typical experiment with 10 dilutions of an antibody is 4 s, whereas the same analysis with a machine without the mathematical coprocessor takes 1 min. Larger experiments require proportionally greater time intervals with the two different types of machines. A free copy of this compiled software program can be obtained from the author.

Curve parameters calculated. The regression analysis was based on the algorithm of Marquardt (7), which provides a compromise between the Taylor series linearization method and that of the steepest descent (gradient method). CRD curves have a plateau zone in which the fraction of the antigen bound does not change and a dissociation zone in which the fraction of the antigen bound is decreased. The definition of these zones is useful to the analysis of the antigen-antibody reaction. The statistical data reported by the program were designed to be comprehensive. Values for the standard deviation, variance, residual sum of squares, covariance matrix of parameters, correlation matrix of parameters, and standard error of the parameters were calculated during each operation with each set of data.

Operation of the program. No special knowledge of computers is required to operate the program, since all commands require only the entry of a number or letter. Numbers are required to select from a menu of items displayed on the screen or to enter a specific value. A letter is entered to answer simple yes-or-no questions. Two different types of analysis are presented for selection in the main menu of the

TABLE 1. AFCRV unimodal sector results^a

Original dilution	Original fraction bound	Estimated fraction bound
1	0.535	0.5440851
0.518	0.495	0.4924089
0.26	0.438	0.4216068
0.131	0.348	0.3381702
0.0978	0.28	0.3005926
0.0674	0.26	0.2528775
0.051	0.172	0.1715603
0.018	0.11	0.1104048
0.00928	0.065	0.06500369

^a Original and calculated values of antibody dilution experiment

TABLE 2. AFCRV unimodal sector results^a

Parameter	Final parameter value (SE)	Coefficient of variation
Binding constant 1	288,936.8 (19,585.92)	6.778617E-02
Antibody concn	2.8886166E-05 (7.560811E-07)	2.617451E-02

^a Calculated values of antibody dilution experiment.

program, one for a unimodal species analysis and the other for a bimodal species analysis.

The unimodal sector evaluates the reaction of a single antibody with a single antigen. The bimodal sector is selected when the total antibody concentration is divided between two different antibody species with different binding constants that compete for a single species of free antigen. The bimodal sector can also be used for the opposite condition, in which there are two species of antigen and only one type of free antibody. After selection of the proper model, the appropriate values for the dilutions, the fraction bound, and the concentration of the original antibody or antigen are entered. If the bimodal sector is selected, an additional value for the estimated distribution constant of the two species of antibody is also entered.

The calculations are all completed without additional data inquiries from the operator to speed up the production of the final data either on the screen or on a hardcopy print via a printer. This program provides the advantage that the complicated analysis is rapidly accomplished in a laboratory with no requirement for the use of a large mainframe computer or complicated statistical software programs.

TABLE 3. AFCRV bimodal sector results^a

Original dilution	Original fraction bound	Calculated fraction bound
1	0.369	0.3547931
0.531	0.32	0.3367743
0.357	0.327	0.3214412
0.274	0.314	0.309484
0.222	0.297	0.2990708
0.188	0.287	0.2903515
0.142	0.265	0.2748562
0.116	0.253	0.2632785
9.619999E-02	0.249	0.2524295
0.0738	0.24	0.2371287
0.0591	0.238	0.2246308
0.0497	0.21	0.2152382
0.0383	0.215	0.2019001
0.0307	0.195	0.1914648
0.0263	0.197	0.1847015
0.02	0.168	0.1738458
0.0161	0.158	0.166246
0.0135	0.176	0.160689
0.00994	0.133	0.1521992
0.00827	0.149	0.1477103
0.00724	0.14	0.1447014
0.00509	0.146	0.1374563
0.00419	0.131	0.133758
0.00257	0.117	0.1247229
0.00236	0.14	0.1231131
0.00221	9.880001E-02	0.1218543
0.00126	0.126	0.1100822
0.00114	0.105	0.107739
0.000633	0.0899	9.217689E-02

^a Original and calculated values of antibody dilution experiment.

TABLE 4. AFCRV bimodal sector results^a

Parameter	Final parameter value (SE)	Coefficient of variation
Binding constant 1	34,108.3 (6,224.724)	0.1824988
Binding constant 2	9,947,209 (4,405,105)	0.4428484
Dissociation constant	0.9441547 (2.045634E-02)	0.0216663

^a Calculated values of antibody dilution experiment.

RESULTS AND DISCUSSION

Affinity binding constants were calculated with data from two different laboratory experiments reported by Nelson and Griswold (9). One set of laboratory data, described as experiment 216-D280 in that paper, was calculated with the unimodal portion of the AFCRV computer program. Table 1 contains the dilutions, the original fraction bound recorded for each dilution, and the fitted values calculated for each dilution of that laboratory experiment. The final binding constant for the single antigen of the experiment is shown in Table 2. These unimodal data represented hyperimmune antiserum taken 280 days after injection.

Data from laboratory experiment 216-D28, which was also reported by Nelson and Griswold (9), were calculated by using the bimodal sector of the AFCRV computer program. The dilutions, the original fractions bound, and the fitted values calculated for each dilution of this bimodal experiment are contained in Table 3. The final binding constants of the high-affinity and low-affinity antibodies are shown in Table 4. The antiserum measured in this experiment was taken 28 days after injection and demonstrates the heterogeneity of the response to the injection of bovine serum at that time.

The calculation and review of CRD curves of antigen-antibody reactions permit a concentrated study of the distribution of affinity throughout a broad range of reactant concentrations while maintaining a constant ratio of the interreactants. The shape of the curve can be derived from the plot of the fraction of the antigen bound versus the dilution factor. The CRD method permits a wide range of dilution factors to be evaluated and was determined to be an improved technique to calculate and study antibody affinity (9). Lowder et al. (5) also have reported the successful use of the CRD technique with monoclonal antibodies. In addition, they reported the comparison of the CRD technique with the equilibrium plate method for these same monoclonal antibodies and found "remarkably close absolute values" for the binding constants from both methods.

The AFCRV computer program can be used in the laboratory in conjunction with the radioimmunoassay by virtually all scientists, including those who have a minimum knowledge of the operation of computers. The program permits rapid calculation of CRD curves and affinity constants in both homogeneous and heterogeneous antigen-antibody mixtures.

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