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The Antiprogesterone Antibody - Progesterone System; RIA Method in Evaluation of Some Kinetic and Thermodynamic Parameters

I. I. DOROBANTU, DELIA-IRINA COROL
National Institute of C&D for Physics and Nuclear Engineering "Horia Hulubei", Environmental and Life Physics Department,
407 Atomistilor St., PO BOX MG 6, R-76900, Bucharest-Magurele

Abstract

The affinity constants, the rate constants of the immunological reactions, avidity (Gibbs energy) and the optimal time to achieve a chemical equilibrum, all these represent the immunogenic qualities of antibodies and antigens and are esential in establishing the optimal conditions for RIA (radioimmunoassay) analysis. The present paper evaluates these parameters for the steroid hormone progesterone byRIA technique which uses as labeled antigens two radioactive markers: progesterone-^{3}H and progesterone-^{125}I.

Keywords: progesterone-^{3}H; progesterone-^{125}I; affinity constant, Gibbs energy, RIA reaction.

Materials and Method

- progesterone-^{3}H (Pr-^{3}H);
- progesterone-6-CMTE-tyramine-^{125}I (Pr-^{125}I);
- progesterone-6-carboxymethylthioether-bovine serum albumine conjugate (Pr-6-CMTE-BSA);
- antiprogesterone-antibodies;

We used in our experiments antiprogesterone-antibodies obtained in our laboratory by the rabbit immunization with progesterone-6-carboxymethylthioether-bovine serum albumine conjugate (Pr-6-CMTE-BSA).

The radioactive markers progesterone-^{3}H and progesterone-6-CMTE-tyramine-^{125}I have also been made in our institute.

Samples of antiprogesterone-antibodies and radioactive progesterone (Pr-^{3}H or Pr-^{125}I) were incubated in the RIA system at different intervals of time. The immune reaction was stopped with ammonium sulphate and the precipitates obtained by centrifugation (in the case of progesterone-^{125}I) or supernatant (for progesterone-^{3}H) were used for the radioactive measurements.

Kinetics of the Antigen-Antibody System

The antiprogesterone antibody – progesterone system (Ab-Pr) represents an example of biomolecular reversibil reaction.

$$Ab + Pr \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} Ab\text{-}Pr$$

where [AbPr] – the concentration of the immune complex;

[Ab] – the concentration of the antibodies;

[Pr] – the concentration of the progesterone of a given time;

K_{+1} – rate constant of forward chemical reaction (the forming constant of the immune complex);

K_{-1} – rate constant of backward chemical reaction (the dissociation constant of the immune complex).

The formation rate (v) for the immune complex (Ab-Pr) will be:

$$v = \frac{d[Ab\text{-}Pr]}{dt} = K_{+1}[Ab][Pr] - K_{-1}[Ab\text{-}Pr] \qquad (1)$$

where [Ab], [Pr], and [Ab-Pr] are the concentrations of the antiprogesterone antibodies, progesterone and, respectively, immune complex, at a given time.

If one notes *[Ab-Pr] = x; [Ab]= a and [Pr]=p*, then it will result:

$$v = \frac{dx}{dt} = K_{+1}a \cdot p - K_{-1}x \qquad (2)$$

After attaining of chemical equilibrum (theoretically, after infinite time), the rate becomes zero and consequently will result:

$$K_{+1}a_e p_e - K_{-1}X_e = 0 \qquad (3)$$

where: a_e , p_e , X_e are concentrations of reactants at equilibrum.

If the value of K_{-1} resulted from (3) is replaced in (2), then:

$$K_{-1} = K_{+1}\frac{a_e p_e}{x_e} \qquad (4)$$

and $$v = \frac{dx}{dt} = K_{+1} \cdot a \cdot p - K_{+1}\frac{a_e p_e}{x_e}x \qquad (5)$$

By replacement of *a* and *p* against initial concentrations of antibodies (a_0) and progesterone (p_0), respectively, results:

$$a = a_0 - x; \qquad p = p_0 - x$$

The reaction rate becomes:

$$v = \frac{dx}{dt} = K_{+1}(a_0 - x)(p_0 - x) - K_{+1}(a_0 - x_e)(p_0 - x_e)\frac{x}{x_e}$$

or

$$v = \frac{K_{+1}}{x_e}(a_0 p_0 - x x_e)(x_e - x) \qquad (6)$$

Therefore,

$$\int \frac{dx}{(a_0 p_0 - x x_e)(x_e - x)} = \int \frac{K_{+1}}{x_e} dt \qquad (7)$$

By integration results:

$$\frac{1}{(-a_0 p_0 + x_e^2)} \ln \frac{(x_e - x)}{(a_0 p_0 - x x_{e)}} = \frac{K_{+1} t}{x_e} + \alpha \qquad (8)$$

where α is a constant of integration, which are determined when t=o, x=o.

$$\alpha = \frac{1}{(-a_0 p_0 + x_e^2)} \ln \frac{x_e}{a_0 p_0} \qquad (9)$$

By transformation of natural logarithm in decimal , (8) becomes:

$$\frac{2{,}303}{(-a_0 p_0 + x_e^2)} \lg \frac{a_0 p_0 (x_e - x)}{x_e (a_0 p_0 - x x_e)} = \frac{K_{+1}}{x_e} t \qquad (10)$$

For rate constant K_{+1}, it was obtained:

$$K_{+1} = \frac{2{,}303 \cdot x_e}{(-a_0 p_0 + x_e^2)} \cdot \frac{1}{t} \cdot \lg \frac{a_0 p_0 (x_e - x)}{x_e (a_0 p_0 - x x_e)} \qquad (11)$$

The rate constants are evaluated easily in the case of using of radioactive labeled progesterone (Pr-^{3}H or Pr-^{125}I), through determination of x and x_e from radioactivity measurements.

$$\frac{x_e}{p_0} = \frac{B_e^s}{T_0} \quad \text{and} \quad \frac{x}{p_0} = \frac{B^s}{T_0} \qquad (12)$$

where:

B_e^s - radioactivity specifically bounded in immune complex at equilibrum;

B^s - radioactivity specifically bounded at time t;

T_0 - total radioactivity introduced in reaction system.

$$K_{+1} = \frac{2{,}303 \dfrac{B_e^s}{T_0}}{-a_0 + \left(\dfrac{B_e^s}{T_0}\right)^2 \cdot p_0} \cdot \frac{1}{t} \lg \frac{a_0}{B_e^s} \cdot \frac{(B_e^s - B^s)}{\left(a_0 - \dfrac{B^2}{T_0}\right)\left(\dfrac{B_e^s}{T_0} - p_0\right)} \qquad (13)$$

Because $p_0 << a_0$ and $B^s/T_0 << 1$, $B_e^s / T_0 << 1$:

$$K_{+1} = \frac{2{,}303\dfrac{B_e^s}{T_0}}{a_0} \cdot \frac{1}{t} \cdot \lg \frac{B_e^s}{(B_e^s - B^s)} \qquad (14)$$

$$K_{-1} = K_{+1} a_0 \left(\frac{T_0}{B_e^s} - 1 \right) \qquad (15)$$

The Affinity and Avidity (Gibbs Energy) of the Antigen-Antibody System

Affinity is the property of the antigen and represents value of the equilibrum constant (affinity constant), K, whereas the avidity is a characteristic of the antibody which is defined as the energy of the antibody for its antigen and is numerically equal to the energy of bonding between antigen and antibody. [1-5], [6].

The antigen - antibody reaction obeys the Law of Mass Action which states that, at equilibrum, the ratio of the concentration's products on the two sides on equation will be constant. Therefore, at chemical equilibrum, in the case of antiprogesterone-antibody system, then:

$$\frac{K_{+1}}{K_{-1}} = K = \frac{[Ab\text{ - }Pr]_e}{[Ab]_e [Pr]_e} \qquad (16)$$

where $[Ab\text{-}Pr]_e$, $[Ab]_e$, $[Pr]_e$ – the concentrations of the immune complex, the antiprogesterone-antibodies and progesterone, after the equilibrum had been reached.

K_{+1} and K_{-1}, represents the rate of association and dissociation constants, K is the equilibrum constant which can be calculated according to (3) and (5).

$$K = \frac{x_e}{(a_0 - x_e)(p_0 - x_e)} = \frac{K_{+1}}{K_{-1}} \qquad (17)$$

The value of x_e is obtained from (12) and it results:

$$K = \frac{B_e^s}{T_o - B_e^s} \cdot \frac{1}{a_0 - \dfrac{B_e^s}{T_0} \cdot p_0} \qquad (18)$$

where a_0 and p_0 represents the initial concentrations of the antiprogesterone antibodies and the progesterone, respectively, B_e^s and T_0 are the specific bound radioactivity at equilibrum and the total radioactivity introduced into the system, K is the affinity constant expressed in liter x mol^{-1}.

The Avidity (Gibbs Energy) of the Antiprogesterone Antibody-Progesterone System

For a reversible chemical reaction at equilibrum the value of the standard reaction Gibbs energy [8] is given by the relation:

$$\Delta G = -2.3\ RT\ \lg K_e \qquad (19)$$

where K_e represents the equilibrum constant

R is the gas constant (R=8.31 joule x $^0K^{-1}$ x mol^{-1})

T is the absolute temperature reported in Kelvin degrees (^0K).

By replacement of (18) in (19) for the antiprogesterone anibody-progesterone system, results:

$$\Delta G = -2.3\ RT\ \lg K_e = -2.3\ RT\ \lg \left| \frac{B_e^s}{T_o - B_e^s} \cdot \frac{1}{a_0 - \frac{B_e^s}{T_0} \cdot p_0} \right| \qquad (20)$$

The value of the avidity (Gibbs energy) is given in Joule x mol^{-1}.

Results and Discussion

Samples of antiprogesterone antibodies (100 μl) and radioactive progesterone (50μl) were completed up to 1 ml with 0.05 M phosphate buffer, pH=7.2 (containing horse serum 10 mg/ml) and incubated at different interval of time.[7] The immune reaction was stopped with 1 ml of 50% ammonium sulphate and the precipitate (which contains the radioactive immune complex) was separated by centrifugation at 1500 g for 30 minutes. The supernatant was collected (for samples which contain progesterone-^{3}H) and measured at β-counter (52% efficiency) using scintillation liquid.

Also the precipitate (in the case of the sample that contains progesterone-^{125}I) was measured at γ-counter (83% efficiency for ^{125}I).

In other experiments samples of antiprogesterone antibodies, radioactive progesterone (progesterone-^{3}H) and different concentrations of nonradioactive progesterone were incubated for 2 hours at room temperature (23^0 C).

Samples were then precipitated with ammonium sulphate and centrifuged in order to separate the precipitate which contains radioactive and nonradioactive complex.

Supernatant fraction was introduced into a scintillation vial containing scintillation liquid suitable for acqueous materials.

Table 1. Kinetics of the antiprogesterone antibody-progesterone system
Radioactive marker Progesterone-^{3}H
T_0 = 17 500 ipm
a = 1.7 x 10^{-8} M
Working temperature: T = 296^0K (23^0C)

Reaction time (min)	**B^s (ipm)**	**$\frac{B^s}{B_e^s}\cdot 100$ (%)**	**K_{+1} (lxmol^{-1}xmin^{-1})**	**K_{-1} (min^{-1})**	**$K=\frac{K_{+1}}{K_{-1}}$ (lxmol^{-1})**	**ΔG = -2.3 RT lgK (j.mol^{-1})**
5	5912	73.2	$7.1x10^6$			
10	7140	88.4	$5.82x10^6$			
15	7762	96.1	$5.85x10^6$	1.2 x 10^{-1}	$5.06x10^7$	43 585
20	7939	98.3	$5.5x10^6$			(43.6 kJ.mol^{-1})
30	8054	100	-			
60	8037	100	-			
120	8141	100	-			

$B_e^s = (B_{e(30)}^s + B_{e(60)}^s + B_{e(120)}^s)/3$=8077

$\overline{K_{+1}} = 6.07 \pm 0.7 \cdot 10^6\, l \cdot mol^{-1} \cdot \min^{-1}$

Table 2. Kinetics of the antiprogesterone antibody – progesterone system
Radioactive marker Progesterone-6-S-CH_2-CO-Tyramine-^{125}I
T_0 = 94850 ipm
a = 3.4 x 10^{-8} M
Working temperature: T = 296^0K (23^0C)

Reaction time (min)	**B^s (ipm)**	**$\frac{B^s}{B_e^s}\cdot 100$ (%)**	**K_{+1} (lxmol^{-1}xmin^{-1})**	**K_{-1} (min^{-1})**	**$K=\frac{K_{+1}}{K_{-1}}$ (lxmol^{-1})**	**ΔG = -2.3 RT lgK (j.mol^{-1})**
5	14970	66.9	$1.54\ x10^6$			
10	18992	85	$1.32\ x10^6$			
15	20849	93.2	$1.23\ x10^6$	1.45 x 10^{-1}	$9.01x10^6$	39 370
20	21603	96.6	$1.26\ x10^6$			(39.4 kJ.mol^{-1})
30	22408	100	-			
60	22325	100	-			
120	22380	100	-			

$B_e^s = (B_{e(30)}^s + B_{e(60)}^s + B_{e(120)}^s)/3$=22370;

$\overline{K_{+1}} = 1.32 \pm 0.07 \cdot 10^6\, l \cdot mol^{-1} \cdot \min^{-1}$

Table 3. Antiprogesterone antibody-progesterone system
Radioactive marker Progesterone-^{3}H
T_0 = 17 500 ipm
a = 1.7 x 10^{-8} M
Working temperature: T = 296^0K (23^0C)

	Pr-^{3}H (ng)	**Pr. (ng)**	**B_e (ipm)**	B_e^s $(B_e - B_{e.nesp})$ **(ipm)**	$\frac{B_e^s}{T_0}$	$\frac{B_e^s}{T_0 - B_e^s}$	$K = \frac{K_{+1}}{K_{-1}}$ **(lxmol^{-1})**	**ΔG = -2.3 RT lgK (j.mol^{-1})**
Antiserum	0.2	0	10350	8054	0.46	0.85	5.1x10^7	
	0.2	0.5	10009	7713	0.44	0.78	4.8 x10^7	
	0.2	1	9919	7623	0.43	0.75	4.8 x10^7	43026
	0.2	2	8792	6496	0.37	0.59	4.0 x10^7	(43 kJ.mol^{-1})
	0.2	3	7705	5409	0.31	0.45	3.2 x10^7	
	0.2	5	7021	4725	0.27	0.37	2.9 x10^7	
	0.2	10	6321	4025	0.23	0.33	3.4 x10^7	
Normal serum		0	2307	-	-	-	-	-
	0.2	10	2285	-	-	-	-	-
	0.2							

$B^s_{e.nesp} = (2307 + 2285)/2 = 2296$

$\overline{K} = (4.03 \pm 0.89) \cdot 10^6 l \cdot mol^{-1}$

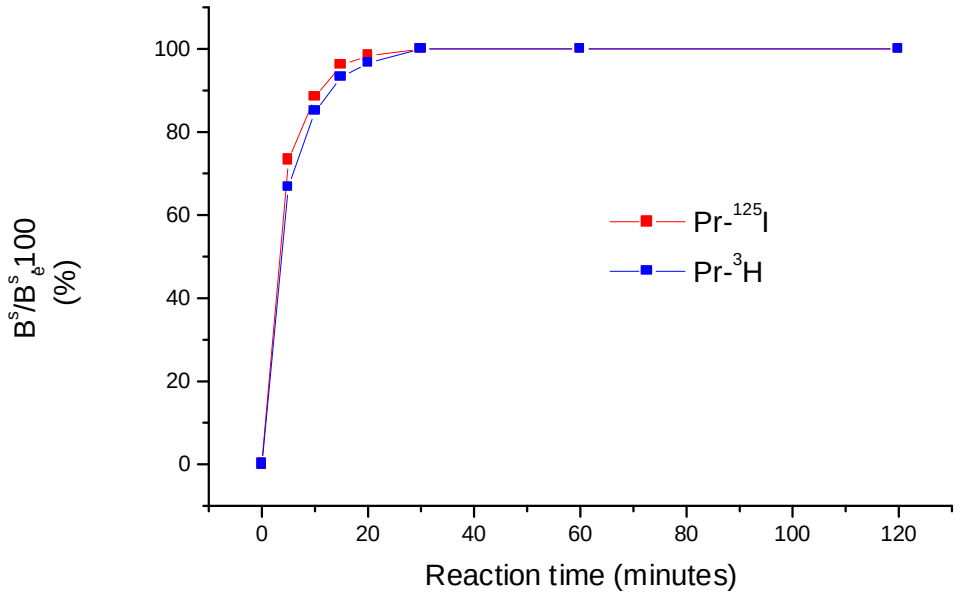

Figure 1. Development of the reaction for antiprogesterone antibody – progesterone system

Conclusion

The measurements of the kinetic parameters K_{+1} and K_{-1} for the progesterone-antiprogesterone antibody in the above exposed conditions lead to an equilibrum constant (affinity constant) of $9.1x10^{6}$ $lxmol^{-1}$, when progesterone-Tyramine-^{125}I was used and $5.061x10^{7}$ $lxmol^{-1}$, in the case of progesterone-^{3}H. For the both systems , the optimal minimal time for reaching the chemical equilibrum was about 30 minutes.

References

1. Knopp D., Glass S. - *Arch. Occup. Environ. on Health*, 63, 5, 329-33, (1991).
2. Blaldey B. R. - *J. Immunopharmacol*., 8, 1, 93-99, (1986).
3. Zhao Y., Li W., Chou In - *J. Toxicol. Environ. Health*, 20, 1, 11-26, (1987).
4. Fleeker J - *J Assoc Off Anal Chem* Sep-Oct 70:5 874-8 *(*1987)
5. Knopp D, Nuhn P, Dobberkau HJ - *Arch Toxicol* Oct 58:1 27-32 (1985)
6. Flanagan RJ, Ruprah M - *Clin Chem Jul* 35:7 1342-7 (1989)
7. Yamada H, Kuroda A, Yatabe T, Inaba T, Chiba K, Iio M - *Radioisotopes* Jun 30:6 311-7(1981)
8. Agasan AL, Stewart BJ, Watson TG - *J Immunol Methods* Dec 28 177:1-2 251-60 (1994)