

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

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### Abstract

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

**Keywords:** progesterone-<sup>3</sup>H; progesterone-<sup>125</sup>I; affinity constant, Gibbs energy, RIA reaction.

### Materials and Method

- progesterone-<sup>3</sup>H (Pr-<sup>3</sup>H);
- progesterone-6-CMTE-tyramine-<sup>125</sup>I (Pr-<sup>125</sup>I);
- progesterone-6-carboxymethylthioether-bovine serum albumine conjugate (Pr-6-CMTE-BSA);
- antiprogesterone-antibodies;

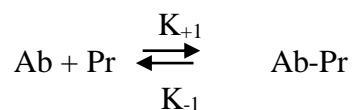
We used in our experiments antiprogesterone-antibodies obtained in our laboratory by the rabbit immunization with progesterone-6-carboxymethylthioether-bovine serum albumine conjugate (Pr-6-CMTE-BSA).

The radioactive markers progesterone-<sup>3</sup>H and progesterone-6-CMTE-tyramine-<sup>125</sup>I have also been made in our institute.

Samples of antiprogesterone-antibodies and radioactive progesterone (Pr-<sup>3</sup>H or Pr-<sup>125</sup>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-<sup>125</sup>I) or supernatant (for progesterone-<sup>3</sup>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 reversible reaction.



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:

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

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  $[\text{Ab-Pr}] = x$ ;  $[\text{Ab}] = a$  and  $[\text{Pr}] = p$ , then it will result:

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

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

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

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

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

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

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

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

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

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

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

Therefore,

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

By integration results:

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

where  $\alpha$  is a constant of integration, which are determined when  $t=0$ ,  $x=0$ .

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

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

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

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

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

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

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

where:

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

$B^s$  - radioactivity specifically bounded at time  $t$ ;

$T_0$  - total radioactivity introduced in reaction system.

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

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

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

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

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

Affinity is the property of the antigen and represents value of the equilibrium 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 equilibrium, the ratio of the concentration's products on the two sides on equation will be constant. Therefore, at chemical equilibrium, in the case of antiprogesterone-antibody system, then:

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

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

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

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

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

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

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 equilibrium and the total radioactivity introduced into the system,  $K$  is the affinity constant expressed in liter x mol<sup>-1</sup>.

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

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

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

where  $K_e$  represents the equilibrium constant

$R$  is the gas constant ( $R=8.31 \text{ joule} \times \text{K}^{-1} \times \text{mol}^{-1}$ )

$T$  is the absolute temperature reported in Kelvin degrees ( $^{\circ}\text{K}$ ).

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

$$\Delta G = -2.3 RT \lg K_e = -2.3 RT \lg \left| \frac{B_e^s}{T_o - B_e^s} \cdot \frac{1}{a_o - \frac{B_e^s}{T_o} \cdot p_o} \right| \quad (20)$$

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

## Results and Discussion

Samples of antiprogesterone antibodies (100  $\mu\text{l}$ ) and radioactive progesterone (50 $\mu\text{l}$ ) 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- $^3\text{H}$ ) and measured at  $\beta$ -counter (52% efficiency) using scintillation liquid.

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

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

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 aqueous materials.

**Table 1.** Kinetics of the antiprogesterone antibody-progesterone system  
 Radioactive marker Progesterone-<sup>3</sup>H  
 $T_0 = 17\,500$  ipm  
 $a = 1.7 \times 10^{-8}$  M  
 Working temperature:  $T = 296^0\text{K}$  ( $23^0\text{C}$ )

| Reaction time (min) | $B^s$ (ipm) | $\frac{B^s}{B_e^s} \cdot 100$ (%) | $K_{+1}$ ( $\text{l}\cdot\text{xmol}^{-1}\cdot\text{xmin}^{-1}$ ) | $K_{-1}$ ( $\text{min}^{-1}$ ) | $K = \frac{K_{+1}}{K_{-1}}$ ( $\text{l}\cdot\text{xmol}^{-1}$ ) | $\Delta G = -2.3 RT \lg K$ ( $\text{j}\cdot\text{mol}^{-1}$ ) |
|---------------------|-------------|-----------------------------------|---|--------------------------------|---|---|
| 5                   | 5912        | 73.2                              | $7.1 \times 10^6$   | $1.2 \times 10^{-1}$           | $5.06 \times 10^7$  | 43 585<br>(43.6 $\text{kJ}\cdot\text{mol}^{-1}$ )             |
| 10                  | 7140        | 88.4                              | $5.82 \times 10^6$  |                                |   |   |
| 15                  | 7762        | 96.1                              | $5.85 \times 10^6$  |                                |   |   |
| 20                  | 7939        | 98.3                              | $5.5 \times 10^6$   |                                |   |   |
| 30                  | 8054        | 100                               | -   |                                |   |   |
| 60                  | 8037        | 100                               | -   |                                |   |   |
| 120                 | 8141        | 100                               | -   |                                |   |   |

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

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

**Table 2.** Kinetics of the antiprogesterone antibody – progesterone system  
 Radioactive marker Progesterone-6-S-CH<sub>2</sub>-CO-Tyramine-<sup>125</sup>I  
 $T_0 = 94850$  ipm  
 $a = 3.4 \times 10^{-8}$  M  
 Working temperature:  $T = 296^0\text{K}$  ( $23^0\text{C}$ )

| Reaction time (min) | $B^s$ (ipm) | $\frac{B^s}{B_e^s} \cdot 100$ (%) | $K_{+1}$ ( $\text{l}\cdot\text{xmol}^{-1}\cdot\text{xmin}^{-1}$ ) | $K_{-1}$ ( $\text{min}^{-1}$ ) | $K = \frac{K_{+1}}{K_{-1}}$ ( $\text{l}\cdot\text{xmol}^{-1}$ ) | $\Delta G = -2.3 RT \lg K$ ( $\text{j}\cdot\text{mol}^{-1}$ ) |
|---------------------|-------------|-----------------------------------|---|--------------------------------|---|---|
| 5                   | 14970       | 66.9                              | $1.54 \times 10^6$  | $1.45 \times 10^{-1}$          | $9.01 \times 10^6$  | 39 370<br>(39.4 $\text{kJ}\cdot\text{mol}^{-1}$ )             |
| 10                  | 18992       | 85                                | $1.32 \times 10^6$  |                                |   |   |
| 15                  | 20849       | 93.2                              | $1.23 \times 10^6$  |                                |   |   |
| 20                  | 21603       | 96.6                              | $1.26 \times 10^6$  |                                |   |   |
| 30                  | 22408       | 100                               | -   |                                |   |   |
| 60                  | 22325       | 100                               | -   |                                |   |   |
| 120                 | 22380       | 100                               | -   |                                |   |   |

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

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

**Table 3.** Antiprogesterone antibody-progesterone system  
Radioactive marker Progesterone-<sup>3</sup>H

T<sub>0</sub> = 17 500 ipm

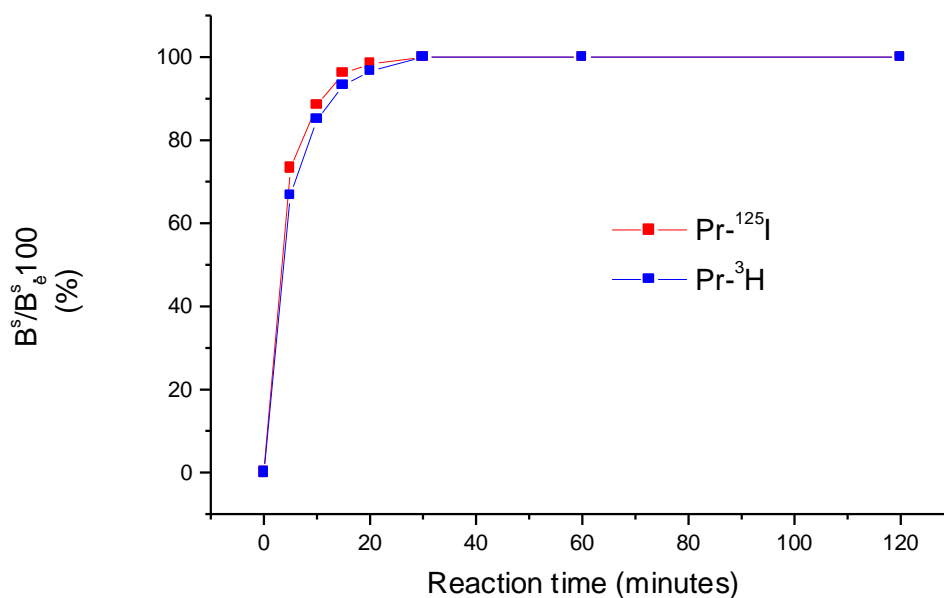
a = 1.7 x 10<sup>-8</sup> M

Working temperature: T = 296<sup>0</sup>K (23<sup>0</sup>C)

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

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

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



**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-antiprogestosterone antibody in the above exposed conditions lead to an equilibrium constant (affinity constant) of  $9.1 \times 10^6 \text{ l mol}^{-1}$ , when progesterone-Tyramine- $^{125}\text{I}$  was used and  $5.061 \times 10^7 \text{ l mol}^{-1}$ , in the case of progesterone- $^3\text{H}$ . For the both systems, the optimal minimal time for reaching the chemical equilibrium was about 30 minutes.

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