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IJPBS |Volume 2| Issue 4 |OCT-DEC |2012|284-286
Letter To Editor
Biological Sciences

PROTEIN – LIGAND BINDING

(Precaution to be taken to avoid the occurrence of wrong result)

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ABSTRACT

The dissociation of a protein – ligand complex (PL) can be described by a simple equilibrium reaction PL ⇌ P + L the corresponding equilibrium relationship is defined K [PL] = [P] [L] (K = dissociation constant) In this equation [P] = $[P]_T$ – [PL] and [L] = $[L]_T$ – [PL] where $[P]_T$ and $[L]_T$ are the initial total concentrations of the protein and ligand, respectively. ***Case1:*** *If we substitute $[L]_T$ – [PL] for [L] and $[P]_T$ – [PL] for [P], then equilibrium relationship becomes K [PL] = ($[L]_T$ – [PL]) ($[P]_T$ – [PL]) From this it follows that [PL] = $[L]_T$[P] / K + [P]* ***Case2:*** *If we substitute $[L]_T$ – [PL] for [L], $[P]_T$ – [PL] for [P] and $[P]_T$ – [P] for [PL], then equilibrium relationship becomes K ($[P]_T$ – [P]) = ($[L]_T$ – [PL]) ($[P]_T$ – [PL])From this it follows that K – [L] = K F_{FP} – F_{BP} [L] (wrong result)* ***Conclusion:*** *Substitution for '[PL]' along with the substitutions for '[L]' and '[P]' should be avoided in order to prevent the occurrence of wrong result.*

KEYWORDS

Protein –ligand binding, protein - ligand complex, equilibrium reaction, dissociation constant.

CASE 1

Using the equilibrium relationship

K [PL] = [L] [P]and substituting,

$[L]_T$ – [PL] for [L]

$[P]_T$ – [PL] for [P]

Gives:

$K [PL] = \{[L]_T - [PL]\} \{[P]_T - [PL]\}$

$= [L]_T [P]_T - [PL] [L]_T - [PL] [P]_T + [PL]^2$

Dividing throughout by [PL] gives:

$K = \{[L]_T [P]_T / [PL]\} - [L]_T - [P]_T + [PL]$

But

$[P]_T = [PL] + [P]$

And, therefore,

$K = \{[L]_T [P]_T / [PL]\} - [L]_T - [P]$

$= [L]_T (\{[P]_T / [PL]\} - 1) - [P]$

From this it follows that

$K + [P] = [L]_T [P] / [PL]$

Rearranging

$[PL] = [L]_T [P] / K + [P]$ (1)

DISCUSSION

This defines a rectangular hyperbola with several important regional properties:

1. Saturation: when [P] >> K, [PL] asymptotically approaches $[L]_T$.
2. Half-saturation: when [P] = K, [PL] = $[L]_T/2$ - in other word, the dissociation constant is equal to the (free) protein concentration needed to ensure that 50% of the ligand will be bounded.
3. Linearity: when [P] << K, [PL] is ~ proportional to [P] with slope = $[L]_T$/ K.

CASE 2

Using the equilibrium relationship

K [PL] = [L] [P] and substituting,

$[P]_T$ – [P] for [PL] $[L]_T$ – [PL] for [L]

$[P]_T$ – [PL] for [P]

Gives:

$K \{[P]_T - [P]\} = \{[L]_T - [PL]\} \{[P]_T - [PL]\}$

$K [P]_T - K [P] = [L]_T [P]_T - [PL] [L]_T - [PL] [P]_T + [PL]^2$

Rearranging

$K [P]_T - [L]_T [P]_T + [PL] [P]_T = - [PL] [L]_T + [PL]^2 + K [P]$

$[P]_T \{K - [L]_T + [PL]\} = [PL] \{- [L]_T + [PL]\} + K [P]$

Further, if we substitute

$[L]_T = [PL] + [L]$

Then we get

$[P]_T \{K - [PL] - [L] + [PL]\} = [PL] \{-[PL] - [L] + [PL]\} + K [P]$

$[P]_T \{K - [L]\} = - [PL] [L] + K [P]$

Which is the same as:

$[P]_T \{K - [L]\} = K [P] - [PL] [L]$

$K - [L] = K \{[P]/ [P]_T\} - \{[PL]/ [P]_T\} [L]$

Labeling

$[P] / [P]_T$ as F_{FP} (fraction of free protein) and $[PL] / [P]_T$ as F_{BP} (fraction of bound protein) then above expression turn into

$K - [L] = K F_{FP} - F_{BP} [L]$ (2)

DISCUSSION

1. If $F_{FP} = F_{BP}=1$, then the LHS = RHS, and the Eq. (2) is true.
2. If $F_{FP} = F_{BP}\neq 1$, then the LHS ≠ RHS, and the Eq. (2) is invalid.

Let us now check the validity of the condition "$F_{FP} = F_{BP}=1$".

As per the protein conservation law,

$[P]_T = [PL] + [P]$

From this it follows that

$1= F_{BP} + F_{FP}$

If we assume $F_{BP} = F_{FP} =1$, we get:

1 = 2

The condition $F_{FP} = F_{BP} =1$ is invalid, since 1 doesn't = 2.

In fact, the only way it can happen that K – [L] = K – [L] is if both $F_{FP} = F_{BP} =1$. Since $F_{FP} = F_{BP} \neq 1$, Eq. (2) does not therefore hold well.

CASE NOTES

CASE 1

If we substitute $[L]_T - [PL]$ for [L] and $[P]_T - [PL]$ for [P], then equilibrium relationship K [PL] = [L] [P] becomes

$K [PL] = ([L]_T - [PL]) ([P]_T - [PL])$

From this it follows that

$[PL] = [L]_T [P] / K + [P]$

CASE 2

If we substitute $[L]_T - [PL]$ for [L], $[P]_T - [PL]$ for [P] and $[P]_T - [P]$ for [PL], then equilibrium relationship K [PL] = [L] [P] becomes

$K ([P]_T - [P]) = ([L]_T - [PL]) ([P]_T - [PL])$

From this it follows that

$K - [L] = K F_{FP} - F_{BP} [L]$ (wrong result)

CONCLUSION

Substitution for '[PL]' along with the substitutions for '[L]' and '[P]' should be avoided in order to prevent the occurrence of wrong result.

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