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Electrochemical Studies of Paclitaxel Interaction with Tubulin

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Abstract: A highly sensitive linear sweep voltammetric method was developed for the determination of paclitaxel and the mechanism of the binding of paclitaxel to tubulin was studied. Tubulin dimer formed with paclitaxel an electrochemically nonactive complex with a combination ratio of 2:2. Its stability constant was 2.85×10^{22}. So the tubulin dimer had two binding sites for paclitaxel. The experiment showed that the binding sites of paclitaxel to tubulin dimer were different from that of Ca^{2+} to tubulin dimer.

Keywords: Paclitaxel , tubulin, linear sweep voltammetry.

Paclitaxel can stabilize microtubules and induce microtubule assembly, so people are interested in the interaction of paclitaxel with tubulin. This interaction has been studied by fluorometry[1], but the electrochemical method has not been adopted to study it so far.

Pig-brain tubulin was purified by temperature-dependent assemly-disassembly procedure[2], protein concentrations were estimated by Bradford method and its purity was determined by SDS-polyacrylamide gel electrophoresis (≥95 % purity). A stock solution of paclitaxel (1.0×10^{-3} mol/L) in methanol was prepared. A JP303 model polarograph (Chengdu Instrumental Factory) was used.

In 0.086 mol/L B-R (pH=6.50) buffer solution, at 37°C, the derivative peak current is proportional to the concentration of paclitaxel over the range of 1.1×10-7—1.9×10-6 mol/L with the detection limit of 8.0×10-8 mol/L. The peak potential of paclitaxel is –1.43V (vs.SCE). Tubulin is added to the electrolytic cell containing paclitaxel with stirring for 1 min at 37 °C, which results in that the peak current of paclitaxel quickly decreases, illustrating that paclitaxel forms a complex with tubulin dimer. The effect of temperature shows that the increasing of temperature is favorable for the binding of paclitaxel to tubulin dimer. The effect of Ca2+ shows that the binding sites of paclitaxel to tubulin dimer are different from that of Ca2+ to tubulin dimer.

The binding ratio of paclitaxel-tubulin dimer complex was determined by the molar ratio method. Under the given concentration of paclitaxel (or tubulin dimer) and adding the tubulin dimer (or paclitaxel), the ip of paclitaxel decreased (or increased). The binding ratio determined was 1:1. So, $mP + mT = P_mT_m$ (P—paclitaxel , T—tubulin).

$$\beta = \frac{[P_m T_m]}{[P]^m [T]^m} \Rightarrow \frac{1}{\beta} = \frac{[P]^m [T]^m}{[P_m T_m]} \qquad (1)$$

If C_P is the concentration of added paclitaxel, C_T is the concentration of added tubulin dimer, I is the peak current of the paclitaxel, k is the constant, then

$[P_m T_m] = (C_p - I/k)/m$, $[P] = I/k$, $[T] = C_T - m[P_m T_m] = C_T - C_p + I/k$ $\Rightarrow$

$I = k[(C_p - C_T) + [T]]$ and $\frac{1}{\beta} = \frac{m(I/k)^m [(C_T - C_p) + I/k]^m}{C_P - I/k}$

When C_T is fixed and $C_P >> C_T$, , $[T]$ can be neglected. So the relationship betwen I and C_P is approximately a linear. The slope is k. We can obtain m=1, $\beta = 5.7 \times 10^8$; m=2, $\beta = 2.85 \times 10^{22}$; m=3, $\beta = 2.0 \times 10^{36}$ ……Because paclitaxel can bind to two tubulin dimers at least[1], and when m=3 or 4, β is too large, so we take m=2, $\beta = 2.85 \times 10^{22}$. It suggests that one tubulin dimer has two binding sites for paclitaxel.

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