



Biolog. Journal of Armenia, 1 (64), 2012

THE INTERACTION OF ZnTOEPyP4 PORPHYRIN WITH tRNA. THE INFLUENCE OF IONIC STRENGTH

I.V. VARDANYAN

Yerevan State University, Department of Molecular Physics
ishkhan@ysu.am

The interaction of ZnTOEPyP4 porphyrin with tRNA was studied using UV/Vis Spectrophotometry and Circular Dichroism (CD) methods. The influence of ionic strength on these interactions was investigated as well as. The preferable binding mechanism was identified.

Porphyrin s- tRNA - ionic strength

Լուսասպեկտրաչափական և շրջանային դիքրոիզմի մեթոդներով ուսումնասիրվել է ZnTOEPyP4 պորֆիրինի փոխազդեցությունը փՌՆԹ-ի հետ միջավայրի տարբեր իոնական ուժերի դեպքում: Պարզաբանվել է փՌՆԹ-ի հետ ZnTOEPyP4 կապման մեխանիզմը:

Պորֆիրիններ - փՌՆԹ - իոնական ուժ

Методами спектрофотометрии и кругового дихроизма исследовано взаимодействие порфирина ZnTOEPyP4 с тРНК при высоких и низких ионных силах. Установлен механизм связывания ZnTOEPyP4 с тРНК.

Порфирины - тРНК - ионная сила

In this work the influence of ionic strength on the interaction of tRNA from E.Coli with Zn(II) meso-tetra-(4N-oxyethylpyridyl) porphyrin (ZnTOEPyP4) has been studied by UV/Vis Spectrophotometry and Circular Dichroism methods. There are few works devoted to the interaction of porphyrins with tRNA [1, 2], while the interaction of ZnTOEPyP4 with tRNA is not investigated at all. It is known that conformation of tRNA strongly depends on ionic strength of medium. In case of high ionic strength ($\mu=0.2$ M) the molecule of tRNA has tertiary structure alike reversed letter “L” (Fig.1). At low ionic strength ($\mu=0.02$ M) tRNA takes a hairpin form with several short double helical regions and loops [3].

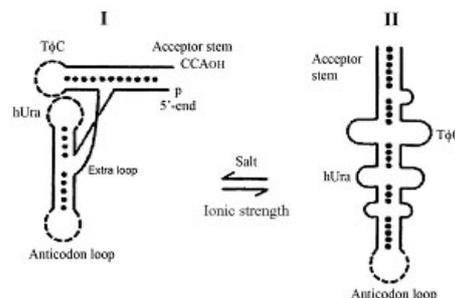


Fig.1. The structure of tRNA at high (I) and low (II) ionic strength

Materials and methods. All measurements were performed in 0.1 BPSE ($\mu=0.02$ M) and 1 BPSE ($\mu=0.2$ M) buffers (1BPSE = 6 mM Na₂HPO₄ + 2 mM NaH₂PO₄ + 185 mM NaCl + 1 mM Na₂EDTA) pH 6.57. ZnTOEPyP₄ was synthesized by Dr. R.Ghazaryan Faculty of General and Nonorganic Chemistry, Yerevan State Medical University.

Results and Discussion. The interaction of porphyrins has been monitored with visible absorption spectroscopy (Soret band). The solutions of constant concentration of porphyrins (10^{-6} M) titrated with a stock solution of tRNA. The results obtained from titration experiments were used for the calculation of the binding parameters. As it is suggested by Cantor and Schimmel the model for binding of ligands with tRNA is described via Scatchard model.

$$\frac{r_b}{C_f} = \frac{N_1 K_1}{1 + C_f K_1} + \frac{N_2 K_2}{1 + C_f K_2}$$

where C_f is the free porphyrins concentration in solution, $r = C_b/C_{tRNA}$, C_b is the concentration of bound porphyrins, C_{tRNA} is the concentration of tRNA molecules. It is supposed, that there are two types of binding sites on tRNA, N_1 sites with binding constant K_1 and N_2 sites with binding constant K_2 .

The binding parameters for mentioned cases are the following:

$$K_1=8.07 \times 10^9 \text{ M}^{-1}, N_1=24.7; K_2=2.09 \times 10^6 \text{ M}^{-1}, N_2=6.9 (\mu=0.02 \text{ M})$$

$$K_1=5.15 \times 10^7 \text{ M}^{-1}, N_1=2.2; K_2=4.03 \times 10^4 \text{ M}^{-1}, N_2=38 (\mu=0.2 \text{ M})$$

From binding parameters it can be seen, that in both cases of ionic strength there is a preferable binding mechanism. Having one axial ligand, ZnTOEPyP₄ cannot intercalate into helical sites, it binds with tRNA externally ordered. Also Zn metalloprophyrin binds with tRNA well in case of low ionic strength (corresponding binding constant is bigger).

As it is known the ICD spectra of complexes of porphyrins with nucleic acids are very informative. A rather unusual effect of ICD band modification is observed when ZnTOEPyP₄ binds with tRNA in both cases of ionic strength (fig.2). From obtained data we can conclude following.

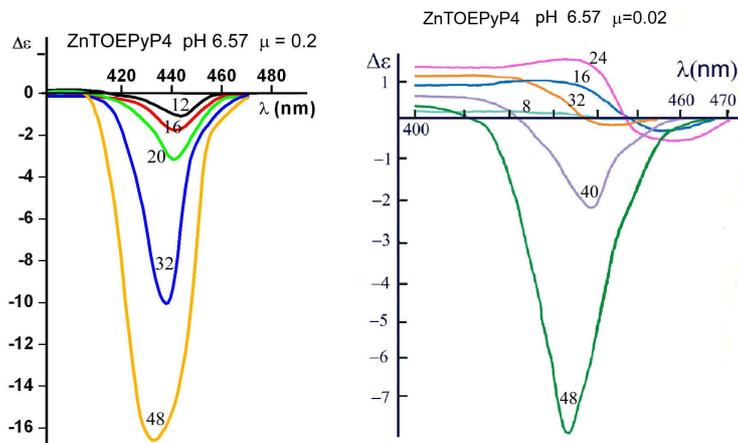


Fig.2. ICD-spectra of complexes ZnTOEPyP₄ with tRNA in case of different ionic strength. Numbers near the curves show the ratio of porphyrins to tRNA

In case of low ionic strength it is proposed that Zn porphyrins are ordered in a stack, not only on helical sites, but also on loops of tRNA [4]. As a result of such ordered stacking, the complex resembles a short stick. These sticks can interact with each other via bound porphyrins. As a result they can form the structures that look like liquid crystals which can show the similar behavior of ICD spectra.

At high ionic strength tRNA has tertiary structure, which looks like reversed letter “L”. In this condition the intensity of ICD spectra is about 2 times bigger. We assume that this kind of effect cannot be explained in terms of single molecule and it is proposed that one type of binding is with sites at the top of tertiary structure of tRNA and the other one is the intermolecular interactions via bound porphyrins on tRNA. And the growing of intensity of ICD is the result of initially stable tertiary structure of tRNA. As a result, we suppose, that the regular structures like liquid crystals are formed.

REFERENCES

1. *Foster N., Singhal A., Smith M., Marcos N., Schray K.*, Interactions of porphyrins and transfer RNA, *Biochim. Biophys. Acta*, *950*, p.118-131, 1988.
2. *Birdsall W., Anderson W., Foster N.*, Studies of interactions of porphyrins with transfer RNA by high-resolution NMR, *Biochim. Biophys. Acta*, *1007*, 176-183, 1989.
3. *D.M. Crothers, P.E. Cole, C.W. Hilbers, and R.G. Shuman.* The molecular mechanism of thermal unfolding of E. coli formylmethionine transfer RNA, *J Mol. Biol.*, *87*, 63-88 1974.
4. *Y. Dalyan, I. Vardanyan, A. Chavushyan, G. Balayan,* Peculiarities of Interaction of Porphyrins with tRNA at Low Ionic Strength, *J. of Biomol. Struct. & Dynamics*, *28*, 123-131, 2010.

Received 18.01.2012