Studies On the Effect of Phosphorylation on the Dipeptides Actions by Radiation Chemistry

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The electron transfer within several dipeptides and their corresponding phosphorylated dipeptides was studied by electron pulse radiolysis, laser photolysis and electron spin resonance. The electron transfer rate constants were calculated by data modeling and kinetic analysis. It is found that the phosphoryl group in peptides participates the electron transfer process, and reduces the electron transfer rate in all cases. These are very important in life science since every biological process refers to the phosphorylation and nonphosphorylation of protein. It may be concerned in personalities and individualities of the personae.

Life science of today has been developed into a molecular level, even an electron level. One of the key points of molecular biology is to clarify the relationship between the vital molecular structure and its function. Of late years. It has been found[1-5] that protein reversible phosphorylation associates with diverse biological processes such as transfer of extracellular signals (hormones, neurotransmitter, nerves impulse and cytokinesis), interaction of proteins, regulation of genes expression, adjustment of enzyme activity, cell proliferation and malignant transformation, so much as with decrepitude, because the phosphate ester acts as energy-reserver and energy-releaser in biological systems. However, nobody knows that how is the action process occurred in it up to the present.

For the purpose to clarify the real action processes of the phosphoryl group in protein chains in biological systems, we comparably studied the electron transfer (ET) within several dipeptides and their corresponding phosphorylated dipeptides synthesized in our laboratory by ZHAO's method[6] as the model of protein, because aminoacids and dipeptides are elementary units in protein structure, by the methods of electron pulse radiolysis, laser photolysis and ESR. These dipeptides and phosphorylated dipeptides are:

IPr-Trp-Tyr **IPr-Tyr-Trp** **IPr-Phe-GlyOEt**
Trp-Tyr **Tyr-Trp**
NDM-TrpOMe **NDT-MetOMe**
Met-Trp

(**iPr** symbols $(iC_3H_7O)_2P=O$; **NDM** symbols N-diisopropyloxy phosphonylmethionyl; **NDT** symbols N-diisopropyloxy phosphonyl tryptophanyl; **Phe** symbols phenylalanine, **Gly** symbols glycin, **Trp** symbols tryptophan, **Tyr** symbols tyrosine, **Met** symbols Methionine. **Et** symbols ethyl; **Me** symbols methyl)

The ET was observed from the radiolysis and laser photolysis[7] of an aqueous solution containing 0.2 mM dipeptide or phosphorylated dipeptide saturated with N_2O at different pH values. The ET direction in iPr-Trp-Tyr is from Tyr residue to Trp residue cation radical, i. e. the electron is transferred from Tyr residue to Trp residue cation radical along the peptide backbone. Trp-Tyr cann't be initiated by $N_3^{\cdot}$, because it reacted with e_{aq}^-. It implies that phosphoryl radical build up firstly by initiation of $OH^{\cdot}$ or $H^{\cdot}$ created by electron radiolysis of water, then the ET occurred along the dipeptide backbone. This means that the phosphoryl group participated the ET process and could stabilized the Trp residue cation radical.

The participation of phosphoryl group in ET was proved by means of data modeling[8]. The ET along the peptide backbone was observed directly by ESR in Trp-Tyr, iPr-Trp-Tyr and iPr-Phe-GlyOEt.[9] It occurred at

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different temperature from phosphoryl radical to aromatic ring of aminocid and then from phenyl radical in Phe residue transferred at last to peptide backbone.

It witnessed also the existence of the phosphoryl radical in the irradiated phosphorylated peptides and the participation of the phosphoryl group in ET of phosphorylated peptides at first process.

The ET was also observed from Tyr residue to Trp residue in iPr-Tyr -Trp being as the same as in iPr-Trp-Tyr, but the ET rate constants were less than that at radiolysis of corresponding nonphosphorylated peptides.

The ET rate constants under different conditions obtained by the data modeling and kinetic analysis are listed in Table I.

Table I. Comparison of the ET rate constants in phosphorylated and nonphosphorylated dipeptides

pH	Initiator	Peptide	R. C. ($10^4 S^{-1}$)	Peptide	R. C. ($10^4 S^{-1}$)
2	$N_3^{\cdot}$	Trp-Tyr	12.1(20)[10]	iPr-Trp-Tyr	4.1
7	$N_3^{\cdot}$	Trp-Tyr	4.3	iPr-Trp-Tyr	3.5
10	$N_3^{\cdot}$ ($Br_2^{\cdot}$)	Trp-Tyr	3.5	iPr-Trp-Tyr	2.9(2.0)
7	$N_3^{\cdot}$	Tyr-Trp	6.7[11]	iPr-Tyr-Trp	5.8

From the table's data it is clear that phosphorylation of dipeptides in different sequence at N-terminal Trp or at N-terminal Tyr decreases the ET rate within the dipeptide. It may be very important because Tyr kinase cell growth factor is a kind of polypeptide hormone playing an important role in cell proliferation by combining the specific receptor on the surface membrane of target cell, evoking the Tyr kinase on receptor.

In the case of the dipeptides being composed of Met and Trp the ET from Trp residue to the three electron-bonded radicals containing sulfur atom (S∴) was observed in NDM-TrpOMe and NDT-MetOMe aqueous solution at pH = 5 and 10 initiated by ·OH and $Br_2^{\cdot}$ using electron pulse radiolysis.

It has been found that ·OH and $Br_2^{\cdot}$ oxidate the Met site and Trp site in above dipeptides containing sulfur, and three electron-bonded radical and indolyl radical formed simultaneously, then the ET along the peptide backbone via Trp-Met occurred.

The ET rate constants in NDM-TrpOMe and NDT-MetOMe were measured and estimated by data modeling and kinetic analysis. They are showing in Table II.

Table II Comparison of the ET rate constants (or oxidation rate constants) in phosphorylated and nonphosphorylated dipeptides (or aminoacids) containing sulfur atom

pH	Initiator	Peptide	R. C.	Peptide	R. C. ($10^4 S^{-1}$)
5	OH· or $Br_2^{\cdot}$	Met-Trp	—	NDM-TrpOMe	6.9
10(9.4)	OH· or $Br_2^{\cdot}$	Met-Trp	($>10^6 S^{-1}$)[12]	NDM-TrpOMe	8.6
5	OH· or $Br_2^{\cdot}$	Trp- Met	—	NDT-MetOMe	2.9
2	·OH	Met	$7.7\times10^9 M^{-1}S^{-1}$	NDM	$2.0\times10^9 M^{-1}S^{-1}$
7	·OH	Met	$8.5\times10^9 M^{-1}S^{-1}$[13]	NDM	$3.7\times10^9 M^{-1}S^{-1}$

From the data in Table II the ET rate constants of phosphorylated dipeptides NDM-TrpOMe AND NDT-

MetOMe are also less than one of the corresponding nonphosphorylated dipeptide Met-Trp. The oxidation rate constants of NDM are less than Met.

Even the rate constants intermolecular ET initiated by $Br_2^{\cdot}$ and $Cl_2^{\cdot}$ involving tryptophan and phosphoryl and nonphosphoryl methionine are the same as intramolecular ET: Trp/NDM more less than Trp/Met. (initiator $Br_2^{\cdot}$ pH 4.5, $2.89\times10^8 M^{-1}S^{-1}$: $4.7\times10^8 M^{-1}S^{-1}$; pH 1, $5.4\times10^8 M^{-1}S^{-1}$: $1.0\times10^9 M^{-1}S^{-1}$.)[14]

According to all above experimental results it has been found that phosphonyl group plays a very important role in ET and oxidation in phosphorylated peptides. From the existent data it would be going to conclude that phosphorylation of the dipeptides even aminoacids decreases the ET rate constants, changes the selectivity to initiators and increases the stability of peptides radicals in more pH range. These may be very inportant in the life science because almost every biological process refers to the phosphorylation of protein.

The atman of the universe from microcosm to macrocosm substantially is a process of variform energy conversion and energy transfer containing ET. The life phenomenon in the world anytime also is a process of energy transfer containing ET. But the differential of the life phenomena derives from the composition and construction of the every-body itself (themselves), then the being appears in a number of different forms and the human being and life phenomena appears in a number of different states, individualites and pesonalities. Such being the case, one would think that for instance, could those persons who is combustible, highstrung, hotblooded, nervy, even spasmodic, i.e. all tinderboxes aren't lacking in phosphorylated peptides in neurofibril of the body? Could we should not supply them befitting phosphorylated chemicals? And inversely, could those persons who is blinkard, retarded, dopy, dully, even dumpish, i. e. all hyponoias aren't overfilling phosphorylated peptides in nerve fiber of the body? Could we should not supply them apposite dephosphorable chemicls?

It is came to light that the associated anamnesis and learning of fruit flies is concerned with the signal cascade system of cycle adenosine monophosphate (cAMP), because the gene product for dunces is diphosphatase of cAMP which act to learning and anamnesis through three possible paths: cAMP-dependant phosphatifying of proteins, cAMP-dependant gene expression and cAMP-dependant control gate for ion routeway[15]. In addition, It is well known that lecithin being composed of unsaturated fatic acid, phosphate, acetylcholine, glycerin et al having a popular molecular formula $CH_2OR_1 \cdot CH_2OR_2 \cdot CH_2OPO_2OHR_3$ (R_1, R_2 are fatic acids, R_3 choline) is a natural soul ataractic and the lecithin content in brain cells of crackskull is only one half as much as normal personane[16]. These all are concernd with the phosphates of neurofibril in human brain cells. A fortiori, it is deemed of late years that phosphoprotein, cholesterol and glycogen are exhibiting most important action in brain information transmittal and the food not only in more extent affects the brain growth and its fuction evolution, but also can govern the personae mentality and behavior[17]. So that the abovementioned discussion is not only a ratiocination and hypothesis, but also probably a fact, i. e. the individualities and personalities of personae are dependent on the extent of phosphorylation of neurofibril in brain cells of them.

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Reference

[1] Marx, J. L., Science 229, 638-640 (1985)

[2] Marx, J., Science 251, 744-746 (1990)

[3] Halpain, S., Girault, J.A., Greengard, P., Nature 343, 369-375 (1990)

[4] Alemany, S., Mato J. M. and Stralfors, P., Nature 330 (5), 77-79 (1987)

[5] Lu Zhipei, Life Chem. (CN) 11(5), 23-25 (1991)

[6] Zhao Yufen, Ji Gaijiao, Zeng Jianing, Synthesis 6, 444-448 (1988)

[7] WANG Zhi-yong, ZHANG Zhi-cheng , ZHANG Man-wei, et al, CN J. Chemistry 13 (4) , 324-333 (1995)

[8] Wong Tsiyong, Zhang Zhicheng, Zhang Manwei, CN J. Chemical Physics 6 (4), 335-341 (1993)

[9] Wong Tsiyong, Zhang Zhicheng, Zhang Man-Wei, Acta Physico- Chimica Sinica 11(4) , 375-379 (1995)

[10] Bobrowski, K., Wierzchowski, K. L., Holeman, J., Ciurak, M., Int , J. Radiat. Biol. 57. 919-932 (1990)

[11] Faraggi, M., Defelippis, M. R. and Klapper, M. H., J. Am, Chem. Soc, 111, 5141-5145 (1989)

[12] Prutz, W. A., Siebert, F., Butler, J., Land, E.J., Menez, A., Garestier T. M., Biochim Biophys, Acta 705, 150-162 (1982).

[13] Adams, G. E., Boag, J.W., Currant , J., et al, Absolute rate constants for the reaction of the hydroxyl radical with organic compounds. Ebert, M., Keene, J.P. Swallow, A.J., et al(eds.) Pulse Rediolysis, New York , Academic Press, 1965, 131.

[14] Chu Gao-sheng, Zhang Zhi-cheng, Zhang Man - Wei et al , J. Radiat. Res. Radiat, Proces. 15 (3), 143-148(1997)

[15] Guo Ai-ke, Chinese Sciene Nens, No. 1227, 3, Mar. 18, (1998)

[16] Ma Liang, Healthful Lecithin, Chinese Publisher for Herbalist Doctor and Galenical, Beijing, (1997).

[17] Cankao Xiaoxi, No. 14843-5, 7, Oct., 5-7, (1999).