D.N.T.Kumar, Nooki Mehdi, Fenglin Huang, Min Zhang, Qufu Wei / International Journal of Engineering Research and Applications (IJERA) ISSN: 2248-9622
www.ijera.com Vol. 2, Issue 4, July-August 2012, pp.077-086

Insight into Fluorine Based Amino Acids,Concepts and Applications

D.N.T.Kumar*[1,2],Nooki Mehdi[3],Fenglin Huang[1],Min Zhang[2,4],Qufu Wei[1]**
[1]Key Laboratory of Eco-Textiles & Laboratory of Nano-Textiles,
[1]Ministry of Education,Jiangnan University Wuxi,214122,P.R.China.
[2]SCME Jiangnan University,Wuxi,214122,Jiangsu Province,P.R.China.
[3]State Key Laboratory of Food Science&Technology,Jiangnan University,Wuxi,214122,P.R.China.
[4]Institute for Applied Chemistry,University of Rostock,Rostock,Germany.

Abstract:

New synthetic methods and fluorinated reagents have been developed,that facilitate the introduction of fluorine or fluorocontaining units into small building blocks, designer compounds, and materials of new and often surprising properties. However,in spite of all these advances, we are still lacking sufficient insight into the fundamental aspects of fluoro-organic chemistry,particularly in the areas of structural, chemical, physicochemical, and biological properties of fluorine-containing compounds.We are interested in this review in evaluating some promising applications of Fluorine based bio compounds based on Amino acids useful in the Life Sciences domain.

Key words: Fluoro-organic chemistry,Amino acids,metabolism,control release systems,probes and sensors.

3 FLUORINATED AMINO ACIDS

Introduction

"The story of amino acid production started in Japan in 1908,when the chemist Dr K.Ikeda, was working on the flavouring components of kelp.The specific taste of the kelp preparations, kombu and katsuobushi, is traditionally very popular with the Japanese[1-12].Although we are studying only about 20 amino acids, there are about six more found in the body". Many others are also known from a variety of sources. Amino acids are the building blocks used to make proteins and peptides. Different amino acids have interesting properties,because they have a variety of structural parts,which result in different polarities and solubilities.[15-35] Each amino acid has at least one amine and one acid functional group as the name implies. Figures 1-3 provide,basis to note some or all of these points. The different properties result from variations in the structures of different "R" groups. The "R" group is often referred to as the amino acid "side chain"."Fluorine has quite unique properties: Its electronegativity is the largest among all elements and its ionisation potential is also the largest,with the exception of helium and neon. Consequently,fluoro-organic chemistry is quite different from the chemistry of the other halogens".[12-39]

4 FLUORINATED AMINO ACIDS

"In recent years major advances have been achieved in fluoro-organic chemistry.Organo-fluorine chemistry has made a remarkable progress and a marked impact on the design and synthesis of a large variety of biologically active molecules such as amines,aminoacids,peptides and other natural products.Naturally occurring amino acids play a pivotal role in living systems and therefore synthetic fluroine containing amino acids have a significant place in the minds of researchers working towards the comprehensions and modification of physiological processes".[11-40]

The introduction of fluorine into organic molecules is widely practised,particularly when tuning the properties of molecules for specialist functions. Fluorine substitution finds a prominent role in pharmaceutical development, in governing bioactivity in a wide range of agrochemical products, in soft materials chemistry such as liquid crystals, in photoresist polymers, self assembling monolayersand in positron emission tomography. Allied to this breadth of activity,is a steady development,in the number and range of fluorination reagents and methods.[3-23]

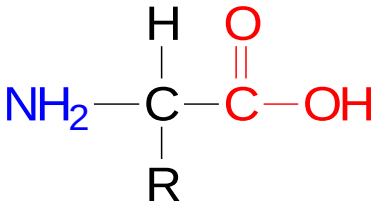

Figure1: Amino acids are named as such because each amino acid consists of an amine portion and a carboxylic acid,as seen above.[5][6][14]permission requested

Figure 2: Amino Acids with alpa,beta,delta,epsilon positions etc...permission requested [12-16]

5 FLUORINATED AMINO ACIDS

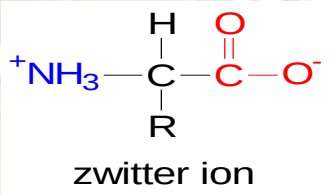

Figure3: Zwitter ion [9]
Permission requested

Among various organofluorine compounds,fluorinated amino acids have been studied as potential enzyme inhibitors and therapeutic agents."Recently, in amino acid and peptide chemistry, amino acids possessing two fluorine atoms at the carbon atom,have been paid much attention because they can act as potent "inactivators", of certain enzymes,in particular,highly selective inhibitors of pyridoxalphosphate dependent enzymes,via a suicide-type mechanism, and can block certain important metabolic pathways.To date,despite the potent biological activities of fluorinated amino acids,very few examples have been reported. Towards further investigation,we highlight some of the bio-chemical material systems,that are described in the following passages".[19-28]

1.1Fluorine Containing Aromatic Amino Acids

"Aromatic alpha-amino acids that are modified at the Beta-position are of interest,as compounds that have influence on the metabolism of serotonin and catecholamines,in the central nervous system. When studying this, we mention here of beta-polyfluoromethyl substituted derivatives of phenylalanine and tyrosine by reduction of the corresponding oxazolones .The latter were
prepared by the Erlenmeyer synthesis from polyfluoromethyl aryl ketones and hippuric acid".[16-35]

6 FLUORINATED AMINO ACIDS

"At present, substitution of hydrogen atoms for fluorine in molecules of naturally occurring compounds is particularly attractive for the construction of low-molecular bio-regulators with a set of required properties that are necessary for the preparation of highly effective pharmaceutical preparations". Figures 4-6 provide,a useful insight into this aspect. Introduction of a fluorine atom instead of hydrogen in molecules of naturally occurring compounds,does not substantially change their spatial structure and their dimensions.[20-29]Consequently, as a rule, enzymes and other bio-polymeric receptors accept molecules with such changes as natural substrates. Then there can also appear "special" properties of the fluorine atom or polyfluorinated groups caused by its high electro negativity.[1,2,16]This stimulated researchers some years ago to approach the realization of a systematic

program,for the investigation into the construction of fluorine containing bio-regulators,that are analogs of naturally occurring compounds.[16]

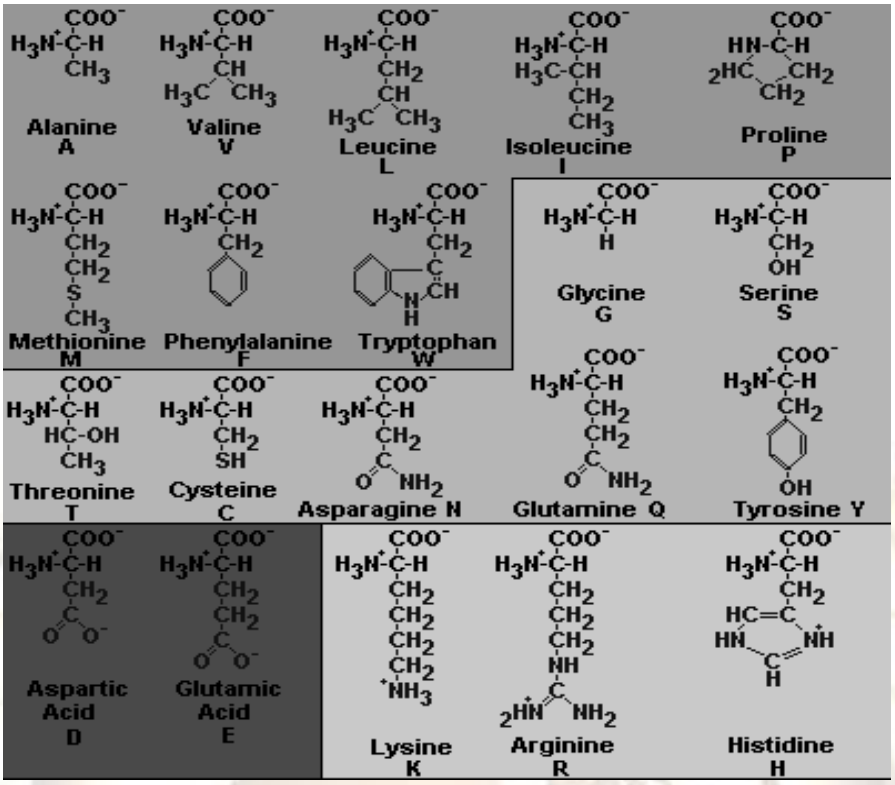

Figure 4: Amino acids and their different groups[Permission requested][33][13]

7 FLUORINATED AMINO ACIDS

1.2 Fluorinated Amino Acids As Bio-Physical & Bio-Chemical Probes

Fluorine NMR experiments with a protein containing fluorinated amino acid analogs, can often be used to probe structure and dynamics of the protein as well as conformational changes,produced by binding of small molecules. The relevance of NMR experiments with fluorine containing materials to characterise the corresponding native (non-fluorinated) proteins depends upon the extent to which the characteristics are altered by the presence of fluorine. "The present work to a great extent uses molecular dynamics simulations,to explore the effects of replacement of tryptophan by 6-fluorotryptophan in folate and methotrexate complexes of the enzyme dihydrofolate reductase(DHFR) (Escherichia coli).It is observed that,simulations of the folate-native enzyme complex produce,local correlation times and order parameters that are generally in good agreement with experimental values.[5-19]

To mention further,simulations of the corresponding fluorotryptophan containing system indicate that the structure and dynamics of this complex are scarcely changed by the presence of fluorinated amino acids".Calculations with the pharmacologically important methotrexate-enzyme complex,predict dynamical behavior of the protein,similar to that of the folate complex for both the fluorinated and native enzyme. It thus appears that, on the time scale sampled by these computer simulations,substitution of 6-fluorotryptophan for tryptophan has little effect on either the structures or dynamics of DHFR,in the complexes[12-39].

"Nonsense suppression methodology[stop condon], for incorporating unnatural amino acids into proteins, has enabled a wide range of studies into protein structure and functions,using both *in-vitro* and *in-vivo* translation systems". Although methodological challenges remain, scores of unnatural amino acids have been employed that include,both subtle and dramatic variants of the natural set. A number of insights,that would not have been possible using conventional site directed mutagenesis,have been gained.[13-31]

8 FLUORINATED AMINO ACIDS

One of the most promising applications of the nonsense suppression methodology would appear to be, the site-

specific incorporation of biophysical probes,such as fluorescent groups or spin labels.
To date, though, this approach has only been minimally exploited."The breadth and generality of the nonsense suppression methodology for unnatural amino acid incorporation,are now firmly established. A wide range of amino acids has been incorporated,across a diverse set of proteins using both *in-vitro* and *in-vivo* expression systems".[12-19]

Significant problems have been addressed, producing insights that would have been difficult to gain with more conventional methods. We can anticipate many more uses for the nonsense suppression methodology. Still, challenges remain. "The most severe is the quantity issue,often not a problem when studying ion channels,but a considerable limitation in some other cases".[13-30]

It is further mentioned that"Wilms tumor protein (WT1) is a transcription factor selectively overexpressed,in leukemias and cancers; clinical trials are underway that use altered WT1 peptide sequences as vaccines. Here we report a strategy to study peptide-MHC interactions by incorporating non-natural and photo-reactive amino acids into the sequence of WT1 peptides. Thirteen WT1 peptides sequences were synthesized with chemically modified amino acids (via fluorination and photo-reactive group additions),at MHC and T cell receptor binding positions. Certain new non-natural peptide analogs could stabilize, MHC class I molecules better than the native sequences,and were also able to elicit specific T-cell responses and sometimes cytotoxicity to leukemia cells".[12-29]

9 FLUORINATED AMINO ACIDS

Two photo-reactive peptides, also modified with a biotin handle for pull-down studies, formed covalent interactions with MHC molecules on live cells and provided kinetic data showing the rapid clearance of the peptide-MHC complex. Despite ''infinite affinity'' provided by the covalent peptide bonding to the MHC, immunogenicity was not enhanced by these peptides because the peptide presentation on the surface,was dominated by catabolism of the complex and only a small percentage of peptide molecules,are covalently bound to the MHC molecules. This study shows that non-natural amino acids,can be successfully incorporated into T cell epitopes,to provide novel immunological, bio-chemical and kinetic information.[18-32]

New Fluorine-Labelled Amino Acids act as, NMR Reporters for Structural Peptide Studies according to the research conducted in Universitaat Karlsruhe,Germany,by a researcher ."The new 19F-labels, CF_3-Bpg and CF_3-MePro,were incorporated into a novel cell-penetrating peptide SAP and six 19F- label analogues were synthesized. Their conformational behaviour was investigated, showing that the label CF_3-MePro stabilizes the helical PP II conformation of SAP, while CF_3-Bpg has no influence. The structure, dynamics and the orientation of SAP in lipid membranes,were then studied by solid state 19F-labelled NMR, showing that the peptide prefers a surface-bound PP II helix under certain conditions".[5-40]

10 FLUORINATED AMINO ACIDS

1.3 Fluorine Amino Acids In Protein Design And Engineering

Protein engineering is a field which focuses on the design and construction of proteins,to improve or develop novel properties through mutation, addition, or deletion of amino acids .These proteins provide significant potential,for advances in pharmaceutical and biotechnological industries as well as basic science. "Its goals focus on the development of novel proteins to improve the quality of life. In most cases, protein engineers require a good knowledge of protein features including: conformation, activity, stability, and specificity. Understanding these features provides a solid foundation for protein design. In route of designing these proteins, engineers generally,employ one of the following schemes: rational and irrational design".[14-22]

"Proteins are essential components of synthetic cellular networks. Synthesis of cellular pathways with the ultimate goal of creating functional artificial cells can benefit greatly from the generation of proteins,with desirable properties that can be effectively regulated".[15-19] Proteins capable of functioning independently of the host organism endogenous circuitry,require further developments in nano-bio device design,based on protein machinery.[15-21]
The recent advances in reassigning the genetic code to unnatural amino acids (UAAs) expand the molecular toolbox of protein parts. In fact, "introduction of UAA can impart new functions that are difficult or impossible to create with proteins comprised of the natural 20 amino acid building blocks such as photo-induced switching,IR probe active and redox sensitive proteins. Moreover,proteins bearing UAAs may result in improved properties such as hyper stability and protease resistance. Such features may be useful for tailoring orthogonal proteins in the context of synthetic biological networks".[11-15]

11 FLUORINATED AMINO ACIDS

Selective incorporation of unnatural amino acids into proteins,is a powerful tool for illuminating the principles of protein design. In particular, fluorinated amino acids,have recently emerged as valuable building blocks,for designing hyper stable protein folds, as well as directing highly specific protein–protein interactions.[21-23]

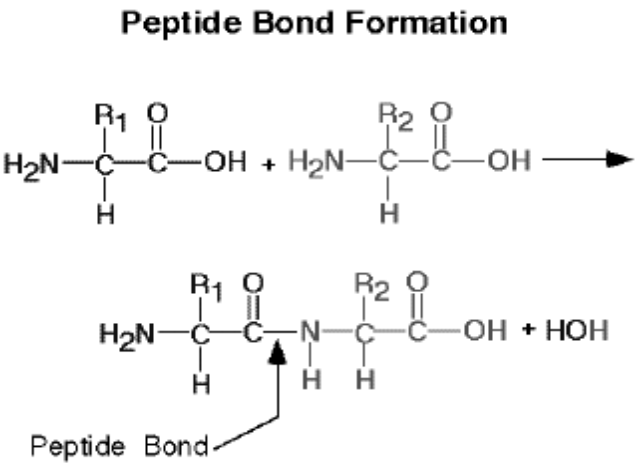

Figure 5:[A]form protein, the amino acids are linked by dehydration synthesis to form peptide bonds. The chain of amino acids is also known as a polypeptide.[Permission requested][15][21][25]

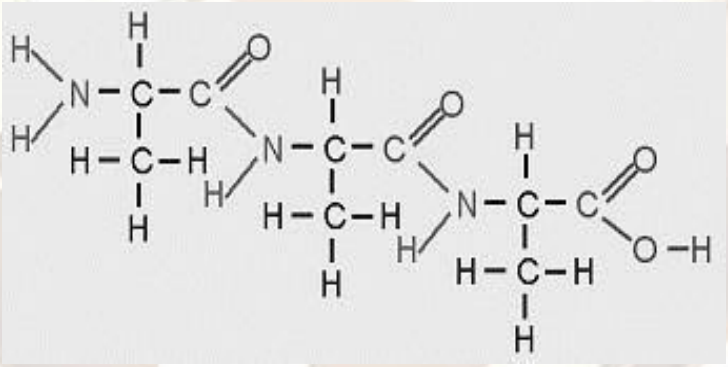

Figure 5:[B]The peptide bonding of three alanine amino acids are shown.
[Permission requested][27]

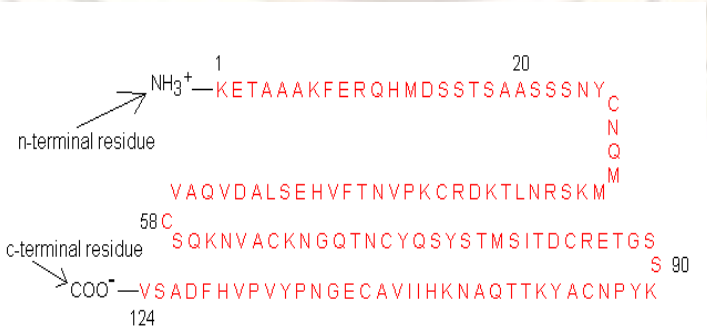

Figure5:[C]A longer polypeptide is shown above. Each peptide chain will have an amine end and a carboxylic acid end and each amino acid is referred to as a residue. So, the ends are named, n-terminal residue and c-terminal residue or the n-terminus and c-terminus.[14]

12 [Permission requested]

1.4 Fluoro-organic Compounds In Non-invasive Medical imaging/Molecular Transport Mechanisms Of Radio-labeled Amino Acids For PET And SPECT

Amino acids are important bio-chemical substrates,that play crucial roles in virtually all biological processes. These ionic nutrients serve not only as basic modules of proteins and hormones, but also as neurotransmitters, synaptic modulators, or neurotransmitter precursors. Transfer of amino acids across the hydrophobic domain of the plasma membrane,is mediated by proteins that recognize,bind, and transport these amino acids from the extracellular medium into the cell, or vice versa.

13 FLUORINATED AMINO ACIDS

In the early 1960s, different substrate-specific transport systems for amino acids in mammalian cells were identified[1-19].General properties of mammalian amino acid transporters were revealed, such as stereospecificity and broad substrate specificity (i.e., several amino acids share the same transport system). Functional criteria such as the type of amino acid (e.g., basic, acidic) or thermodynamic properties(energy dependence of transport) were used to classify amino acid transporters. This classification has been retained to date in functional studies when the molecular mediators of transport are unknown. The molecular identification of amino acid transporters or related proteins started in the early 1990s, and studies on the structure–function relationship and the molecular genetics of the pathology associated with these transporters have generated considerable interest.[12-20]

"The molecular identification of almost all physiologically characterized amino acid transporters in recent years has facilitated the functional analysis of this important class of transport proteins. These studies indicate that although several amino acid transporters are ubiquitous and serve to maintain nutritional demands, many cells additionally express specific transporters that allow the accumulation of particular amino acids". Tumor cells derived from specific cell types often continue to express cell specific transporters and therefore accumulate certain amino acids more than others.[19-22]Radiolabeled amino acids have been considered in nuclear medicine since the early 1960s[1-19]. With the advent of PET, many physiologic amino acids have been radiolabelled by the replacement of a carbon atom by ^{11}C, which does not chemically change the molecule[11].The most frequently used amino acid for PET is,L-[methyl-^{11}C]-methionine(MET), based on convincing clinical results, especially used for the diagnostics of brain tumors, have been reported .

14 FLUORINATED AMINO ACIDS

"It appears that the diagnostic potential of radiolabeled amino acids will be amplified by enlarging knowledge of the molecular mechanisms, involved in the transport processes. The application of radiolabeled amino acids with selective transport characteristics,will offer a new tool for a more specific evaluation of pathologic processes. Furthermore, a new research window may be opened,for the pretherapeutic assessment of antitumor drug selectivity. For instance, the phenylalanine mustard (melphalan) is transported by LAT1 and accumulates in cancer cells"[14-20].The rapidly developing knowledge about the molecular genetics of amino acid transport systems and the capability of nuclear medicine techniques,to measure such processes in humans offer great potential,for improved diagnostics of numerous diseases.[20-22]

1.5 Metabolism Of Fluorine Containing Drugs

Fluorination and metabolism have very strong interaction and co-related bio-chemical effects on the human body.Fluorine substitution in appropriate positions has also been rationalized and exploited to transform a number of amino acids,into inhibitors of their catabolic enzymes.Amino acids containing a trifluoromethyl group have proven therapeutic value as antimetabolites."The relative non-toxicityand stability of Trifluoromethyl compounds compared with mono- and difluoromethyl analogs,make these molecules especially attractive.Uniquely relative to mono- or difluoromethyl groups,the trifluoromethyl group presents, a uniform sphere,of electron-rich fluroines to the enzyme".[12-22]

Anti-Cancer Drugs based on,fluorinated purine and pyrimidine nucleosides (and their nucleotides) have been used,as anticancer drugs since the 1950s. Prominent among them is 5-fluorouracil,a major anti-metabolite used for the treatment of solid cancers. The presence of the fluorine atom at a strategic site prevents addition of formate,which may be necessary for further metabolism. Heidelberger et al. reported in 1957 that tumor cells *in-vitro*,metabolized uracil more efficiently than the healthy cells. This formed the basis for generation of a competitive pyrimidine fluorinated, at the carbon
5-position.[16-21]

15 FLUORINATED AMINO ACIDS

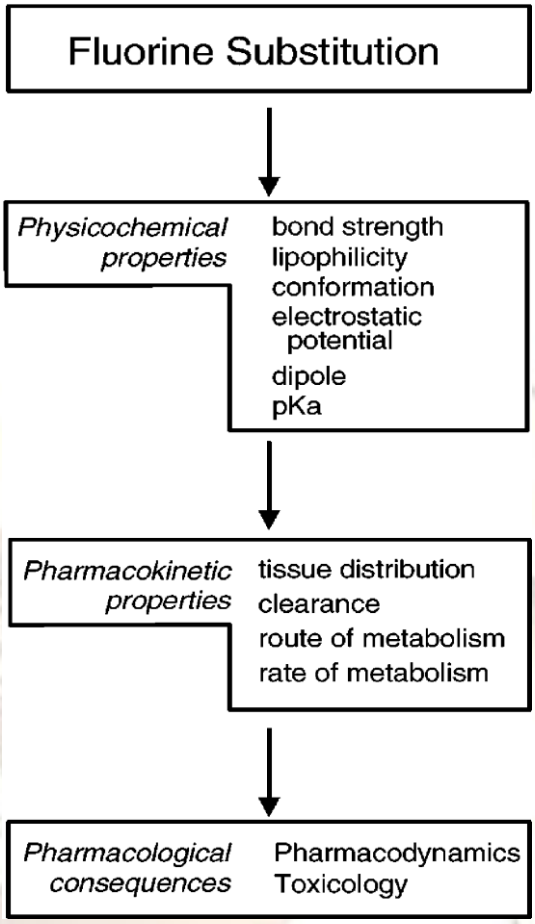

Figure6:Flow diagram illustrating the effect of fluorine substitution on drug response.
[Permission requested][21]

Upon entering the malignant cell 5-fluorouracil gets converted to the active metabolite fluorodeoxyuridine monophosphate (FdUMP) through a series of transformations ."It is believed that 5-fluorouracil,inhibits the action of thymidylate synthase (TS). Thymidyalte synthase is known to play a key role in DNA synthesis, and its inhibition brings about cytotoxicity.Peptides used as therapeutic agents are degraded rapidly by peptidases,explaining the vigorous research conducted,for better non-natural amino acids,with which to substitute natural amino acids". [15-19]

16 FLUORINATED AMINO ACIDS

One strategy is the introduction of fluorine into peptides,to improve the interactions with a recepter site and increase peptide activity.This often produces compounds with reduced metabolism and higher transport rates in-vivo.For example,4-Fluoroglutamic acid(29) was used for synthesis of fluorinated derivatives of 28(X=F).Compound 28(X=F) is methotrexate(MTX) and it is known anti-cancer agent as mentioned in most of the published literatures.[13-20]

Inhalation Anesthetics:

Organofluorine compounds have played a major role,in health care in the form of inhalation anesthetics. Upon inhalation, anesthetics enter the brain and produce various physiological effects like muscle relaxation, sleep and absence of pain, which help facilitate surgery. The evolution of anesthetics, spans a period of nearly150 years. The earliest anesthetics were either based and suffered from one major disadvantage,flammability.[14-21]

Synthetic Blood Substitutes:

In 1966, Leyland Clark discovered that perfluorocarbon (PFC) liquids can dissolve,half their volume of gaseous oxygen, and this led to the basis for fluorinated hydrocarbons being explored as artificial oxygen carriers.[15-17]. "At STP conditions O_2 solubility in PFCs is 45 mL per 100 mL, the CO_2 solubility can be >200 mL.12 Oxygen dissolution in PFCs occurs by O_2 occupying intermolecular

sites in the liquid, unlike the pH and concentration dependent porphyrin binding in hemoglobin of the mammalian blood. The oxygen solubility in PFCs is not sigmoidal, as in blood but increases linearly with increasing partial pressure of O_2" [2-7]

17 FLUORINATED AMINO ACIDS

"Fluorocarbons are passive O_2 carriers and the release and uptake of O_2 is independent of the environment.12 PFCs are unique in their property of having strong intramolecular bonds (covalent) but weak intermolecular bonds (van der Waals interactions), low surface tensions, dielectric constants, gas solubility, hydrophobicity, lipophilicity and chemical inertness.13,14 Fluosol® (Green Cross Corp. Osaka, Japan) was the first PFC emulsion approved by FDA for clinical use as artificial oxygen carrier but did not gain high popularity due to its tedious handling requirements"[5-15]

"Excretion rates of PFCs can be improved,by introduction of lipophilic character in the molecule, a terminal halogen or a short hydrocarbon segment. Oxygent® (Alliance Pharmaceutical Corp., San Diego, US), a 60% w/v F-alkyl bromide emulsion having a small amount of F-decyl bromide, is a promising candidate for O_2 delivery efficiency". However, the Montreal Protocol 7 has restricted production of perhalogenated hydrocarbons since 1996, and their global warming potential coupled with long atmospheric lifetime,may impede a catastrophic PFC boom in the near future[7-21]

18 Conclusions With Future Perspectives

Selective fluorination of biologically active compounds,is often accompanied by dramatic changes in physiological activity. Fluorinated amino acids are being investigated,as pharmaceutical compounds and enzyme inhibitors. Proteins and peptides whose hydrophobic residues are selectively replaced with fluorinated amino acids,show promising trends as industrial enzymes and pharmaceutical proteins with enhanced stability. Many of the unique properties of fluorinated compounds are derived from their unusually strong self-affinity. Selective fluorination of amino acids,can thus provide a specific protein-protein interaction,that is both hydrophobic and lipophobic, and may allow fluorinated peptides,to fold properly in aqueous and organic solvents, as well as within the lipid bilayer of the cell membranes. Fluorinated proteins display an overall increased folding strength, so they are more heat and denaturant resistant than their natural counterparts. More research is being done using this promising technique,to be applied in a wider range of industries all over the world.

Ackowledgements

I thank all our team members and several authors,for encouraging and helping me,to write this review paper in the promising domain of Fluoro-Organic Compounds and related applications.We thank Jiangnan University China,for providing us with the necessary conducive research environment.

19 FLUORINATED AMINO ACIDS

References

[1] V.P. Kukhar ,Fluorine-containing amino acids,http://dx.doi.org/10.1016/0022-1139(94)03131-2.

[2] L.Eggeling et al,Functional Analysis of All Aminotransferase Proteins Inferred from the Genome Sequence ofCorynebacterium glutamicum, J Bacteriol.2005 November;187(22): 7639–7646. doi:10.1128/JB.187.22.7639-7646.2005.

[3] K.Yokota et al,Synthesis, solid-state fluorescence properties, and computational analysis of novel2-aminobenzo[4,5]thieno[3,2-d]pyrimidine 5,5-dioxides,Beilstein J. Org. Chem.2012,8,266–274.published 16 Feb 2012.

[4] Brown et al,"Reactions of Perfluoroalkyl Nitriles.V. Synthesis of Perfluoroacyl Imidates",J. Org.Chem., vol. 30, (1965), pp. 37243728.

[5] Yakubovich et al, "Syntheses in the 1,3,5Triazine Series V. Iminoesters of Perfluorocarboxylic AcidsSynthese,Properties, and Mechanism of Cyclopolymerization to 1,3,5TriazineDerivatives", pp. 878885,(translated from Zhurnal Obshchei Khimii, vol. 36, No. 5, pp 863871,May 1966).

[6] Grinblat et al, "Infrared Investigation of The Vulcanization of Perfluoroalkylenetriazine Polymers", Polymer Science U.S.S.R., vol. 21, 1980, pp. 14341441.

[7] Paciorek et al., "Reactions of Perfluoronitriles. I. Interactions with Aniline", Journal of Fluorine Chemsitry, 30 (1985), pp. 241250.

[8] http://www.patentstorm.us/patents/6794457/claims.html,US PATENT Number :6794457.

[9] Fluorine in the Life Science industryCHIMIA,2004,58,No 3.Editorial.

[10] http://www.halocarbon.com/halocarbon_media/HalocarbonFluoropolymersarticlefor JCT_06_29FINAL.pdf,2006.

[11] B K.Park et al,http://www.fluoridealert.org/f_drugs.pdf , Annu. Rev. Pharmacol. Toxicol. 2001.41:443–70,2001.

[12] D.A. Dougherty,Unnatural amino acids as probes of protein structure and function,Current Opinion in Chemical Biology 2000,4:645–652.

[13] Y. M. Chan et al,Learning Protein Design: Optimization of tGCN5 Progress Report July30,2009.

[14] N Voloshchuk, J Kim & Montclare, Incorporation of unnatural amino acids for synthetic biology,www.rsc.org/molecularbiosystems/Molecular BioSystems.doi: 10.1039/b909200p,2009.20 FLUORINATED AMINO ACIDS

[15] V. P. Kukhar and H. R. Hudson, Aminophosphonic and. Aminophosphinic Acids, Ed.V. P.Kukhar, J. Wiley and. Sons, 2000.

[16] R.Filler&R.Saha,http://www.futuresciencegroup.com/_img/pics/ Fluorine_in_medicinal_chemistry.pdf ,RIIT Chicago,USA.Future Med.Chem2009.1(5).

[17] G. Nuñez et al, (2008) Non-Natural and Photo-Reactive Amino Acids as Biochemical Probes of Immune Function. PLoS ONE 3(12): e3938. doi:10.1371/journal.pone.0003938.

[18] K. H.Piepenbrink et al,Biochem. J. (2009) 423, 353–361 (Printed in Great Britain) doi:10.1042/BJ20090732.

[19] K.J.Langen & S.Bröer,Journal of Nuclear Medicine Vol. 45 No. 9 14351436.

[20] Uchino H, Kanai Y, Kim do K, et al,Transport of amino acidrelated compounds mediated by Ltypeamino acid transporter 1 (LAT1): insights into the mechanisms of substrate recognition. MolPharmacol. 2002;61:729–737.

[21] N.C.Yoder and K.Kumar,Fluorinated amino acids in protein design and engineering,Chem. Soc.Rev.2002, 31, 335341.doi: 10.1039.

[22] P.Mykhailiuk,DISSERTATION,Reporters for Structural Peptide Studies: Design,Synthesis, and Applications (Dr. rer. Nat.) der Fakultät für Chemie und Biowissenschaften der Universität Karlsruhe(TH)vorgelegte von aus DISSERTATION Kerch,Ukraine.10.Juli2008.

[23] http://www.biology.arizona.edu/biochemistry/problem_sets/aa/aa.html#Essentialaa, 2011.

[24] Fluorinated : compatibility with native structures and effects on – interactions Mario Salwiczek ,Elisabeth K. Nyakatura ,Ulla I. M. Gerling ,Shijie Ye and Beate Koksch Chem. Soc. Rev., 2012,41, 2135-2171,DOI: 10.1039/C1CS152

[25] Hammill, J. T.; Miyake-Stoner, S.; Hazen, J. L.; Jackson, J. C.; Mehl, R. A. Nat. Protoc. 2007, 2,2601–

2607. doi:10.1038/nprot.2007.379

[26] Smits, R.; Cadicamo, C. D.; Burger, K.; Koksch, B. Chem. Soc. Rev. 2008, 37, 1727–1739.doi:10.1039/b800310f

[27] Duca, M.; Chen, S. X.; Hecht, S. M. Org. Biomol. Chem. 2008, 6, 3292–3299.doi:10.1039/b806790b

[28] Bilgiçer, B.; Fichera, A.; Kumar, K. J. Am. Chem. Soc. 2001, 123, 4393–4399.doi:10.1021/ja002961j

[29] Chiu, H. P.; Suzuki, Y.; Gullickson, D.; Ahmad, R.; Kokona, B.; Fairman, R.; Cheng, R.P. J, Am. Chem. Soc. 2006, 128, 15556–15557. doi:10.1021/ja0640445

[30] Gottler, L. M.; Salud-Bea, R.; Marsh, E. N. Biochemistry 2008, 47, 4484–4490.doi:10.1021/bi702476

[31] Gerebtzoff, G.; Li-Blatter, X.; Fischer, H.; Frentzel, A.; Seelig, A. ChemBioChem 2004,5, 676–684. doi:10.1002/cbic.200400017

[32] Jäckel, C.; Koksch, B. Eur. J. Org. Chem. 2005, 21, 4483–4503.doi:10.1002/ejoc.200500205

[33] Jäckel, C.; Salwiczek, M.; Koksch, B. Angew. Chem., Int. Ed. 2006, 45, 4198–4203.doi:10.1002/anie.20050438

[34] Salwiczek, M.; Samsonov, S.; Vagt, T.; Nyakatura, E.; Fleige, E.; Numata, J.; Cölfen, H.;Pisabarro, M. T.; Koksch, B. Chemistry 2009, 15, 7628–7636. doi:10.1002/chem.200802136

[36] Lodder, M.; Wang, B. X.; Hecht, S. M. Methods 2005, 36, 245–251.doi:10.1016/j.ymeth.2005.04.002

[37] Choudhury, A. K.; Golovine, S. Y.; Dedkova, L. M.; Hecht, S. M. Biochemistry 2007,46, 4066–4076. doi:10.1021/bi062042

[38] Duca, M.; Chen, S. X.; Hecht, S. M. Methods 2008, 44, 87–99.doi:10.1016/j.ymeth.2007.10.007

[39] Mikami, K.; Itoh, Y.; Yamanaka, M. Chem. Rev. 2004, 104, 1–16.doi:10.1021/cr030685w

[40] Tang, Y.; Ghirlanda, G.; Petka, W. A.; Nakajima, T.; DeGrado, W. F.; Tirrell, D. A.Angew. Chem., Int. Ed. 2001, 40, 1494–1496. doi:10.1002/1521-3773(20010417)40:8<1494::AID-ANIE1494>3.0.CO;2-X