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Review Article

CLICK CHEMISTRY IN NANO DRUG DELIVERY SYSTEM AND ITS APPLICATIONS IN BIOLOGY

Nairrita Majumder*

NSHM Knowledge Campus, College of Pharmaceutical Technology, 124, B.L. Saha Road, Kolkata-700053, West Bengal, India.

ABSTRACT

Since the introduction of the click concept by Sharpless and coworkers in 2001, numerous examples of click reactions have been reported for the preparation and functionalization of polymeric micelles and nanoparticles, liposomes and polymersomes, capsules, microspheres, metal and silica nanoparticles, carbon nanotubes and fullerenes, or bionanoparticles. Among these click processes, Cu(I)-catalyzedazide-alkyne cycloaddition (CuAAC) has attracted most attention based on its high orthogonality, reliability, and experimental simplicity for non-specialists. The alkyne-azidecycloaddition, popularly known as the "click" reaction, has been extensively exploited in molecule/macromolecule build-up, and has offered tremendous potential in the design of nanomaterials for applications in a diverse range of disciplines, including biology. Some advantageous characteristics of this coupling include high efficiency, and adaptability to the environment in which the desired covalent linking of the alkyne and azide terminated moieties needs to be carried out. The efficient delivery of active pharmaceutical agents to specific organelles like mitochondria and lipid bodies, employing nanocarriers developed through the use of "click" chemistry, constitutes a continuing topical area of research. In this review, we highlight important contributions click chemistry in the nanosized drug delivery system and its future aspects.

Keywords: click chemistry, drug delivery, mitochondria, lipid bodies.

INTRODUCTION

In 2001 Sharpless introduced the concept of "click chemistry", one of the most versatile and modular approaches to couple two reactive partners in a facile, quick, selective, reliable and high yield reaction under mild conditions[1]. Since then click chemistry has become one of the most common and reliable methods to link molecules covalently, and it finds applications in a variety of disciplines including the chemistry of nanomaterials, chemical biology, drug delivery, and medicinal chemistry[2–7]. In dendrimer chemistry, CuAAC was used not only for the convergent[8] and divergent build-up[9,10], but also for the dendrimer functionalization, and introduction of multiple functionalities into the macromolecular architecture[11,12–17].

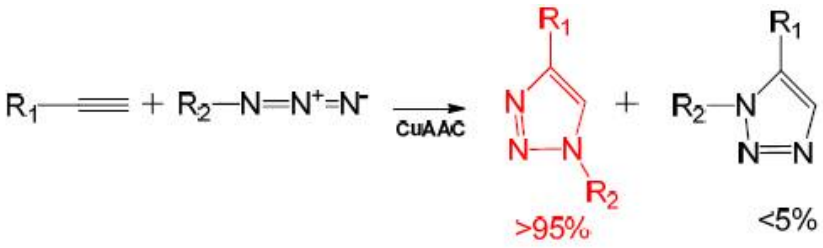
Copper Catalyzed Alkyne-AzideCycloaddition (CuAAC)

The 1,3-dipolar cycloaddition of azides with alkynes was first discovered by Hüisgen in 1963. However, it did not attract much interest until it was demonstrated that this high temperature reaction could also be carried out under mild conditions using Cu(I) as the catalyst, and with tremendous regio-selectivity (Scheme 1). This was discovered simultaneously and independently by Meldal and his group in Denmark, and Fokin and Sharpless in USA[18-21]. The coordination of Cu(I) to alkynes in an

aqueous solution forming a copper-acetylide intermediate is an exothermic reaction. The azide binds to this Cu (I)-acetylide intermediate forming a six membered Cu(III)-metallacycle[22]. Subsequently, the triazole ring formation is very rapid[23], and the cycloaddition product is chemically inert or stable towards redox reactions, has strong dipole moment, hydrogen bond accepting ability and aromatic character[24]. Experimental and computational studies have shown that Cu(I) coordinates to the alkynes through polynuclear Cu(I) intermediates[23,25-29]. Recently, a detailed mechanism has been elucidated by Fokin and his colleagues[30]. The advantages of this alkyne-azide coupling reaction include an almost quantitative conversion, the robust nature of the products, biomolecular ligation, in vivo tagging[31–34], and use in the synthesis of linear polymers[35,36].

Dendrimers

Dendrimers are highly branched macromolecules, which are prepared by repetition of a given set of reactions using either divergent or convergent strategies.[37]Dendrimers consists of three basic architectural components, (i) the core, (ii) the interior and (iii) the end-groups. Generally, the reactions employed are high yielding without any side reactions. This then allows one to obtain defined and uniform structures. Well known processes, such as the Michael reaction, Williamson ether synthesis, amidations and reductions have been used extensively in pioneering work by Vögtle, Tomalia, Fréchet and Newkome.[38]Dendrimers and dendrons can be considered as unique quantized building blocks for nanoscience and have served as functional objects in nanotechnology and nano-materials science.[39,40]. As synthetic nanoscale objects, the structures and properties of the individual dendritic building blocks are hugely versatile. Unlike many other nanoscale objects, dendrimers are inherently synthetically versatile. Therefore dendrimers and dendrons have hugely wide ranging potential applications across a wide range of areas of interdisciplinary science. The two most widely studied dendrimer families are the Fréchet-type polyether and the Tomalia-type poly(amidoamine) (PAMAM) dendrimers.1 The synthetic methods in dendrimer chemistry have recently been upgraded to allow easier access to high-quality dendritic products. These advances have taken advantage of widely-applied approaches such as the click chemistry.[41,42] This has allowed dendritic architectures to be incorporated into ever more elaborate nanostructures.[43]



Scheme 1: Copper-catalyzed alkyne-azidecycloaddition

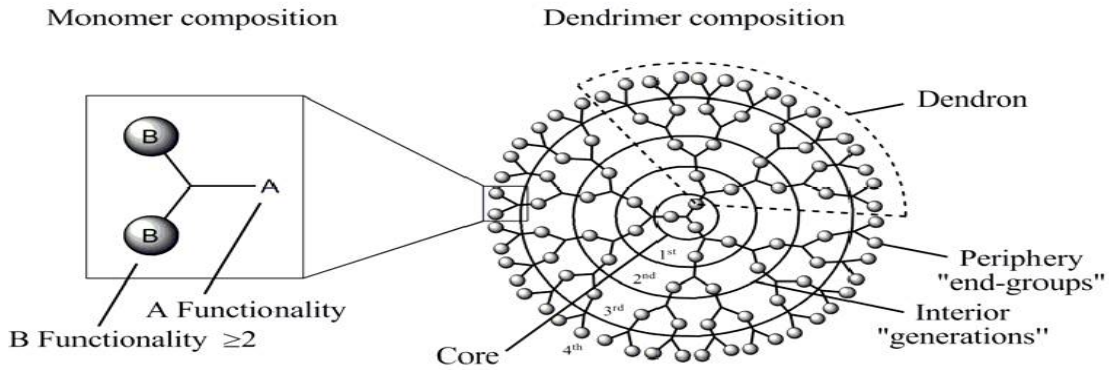


Fig.2:Basic architectural components of a dendrimer

CLICK CHEMISTRY IN NANOSIZED DRUG DELIVERY

Tremendous effort has been devoted to the development of nanocarriers for the efficient delivery of therapeutic agents to the targeted site[44]. In this regard macromolecules have offered tremendous potential[45], but such nanodelivery systems have to meet stringent requirements if they are to be employed for drug delivery[46,47]. The macromolecule based nanocarriers used for this purpose should be non-cytotoxic, remain intact prior to reaching the target site, and enhance the effectiveness of the selected drug. Although, significant efforts have been made in assembling macromolecule based nanocarriers using a variety of synthetic methodologies, challenges still remain in introducing multiple functions into a single platform. Click chemistry has offered new ways of developing nanomaterials[48,49,50], particularly those with multiple functional groups and architecture[51]. These moieties can be introduced within the nanocarrier architecture with high precision. Such nanoarchitectures have been exploited as suitable carriers for therapeutic agents and fluorescent labels to deliver them to specific cells, cellular organelle, to either prevent cell death[52] or visualize them with or without drug delivery. A number of strategies to target cells with drugs had been adopted earlier, these include carbodiimide, thiol-maleimide and biotin-avidin coupling to biomolecules[53]. As already mentioned, recent progress in click chemistry has allowed coupling reactions to be carried out under mild conditions, and in an aqueous medium with negligible unwanted toxic by-products[1].

The use of ‘click’ chemistry to create dendritic modular systems has mainly involved dendrons. ‘Click’ chemistry is a particularly attractive coupling method because it can be performed with a wide variety of solvent conditions including aqueous environments. The stable triazole ring bridge, resulting from coupling alkyne with azide moieties, is frequently achieved at near quantitative yields and is considered to be biologically stable[54-56]. Furthermore, the ‘click’ coupling chemistry is orthogonal to the coupling chemistries typically used to attach functional groups to the dendrimer. Lee and co-workers have detailed the synthesis of multi-module platforms using both un-functionalized PAMAM dendrons[57-59] as well as unfunctionalizedFrechet-type dendrons[60] for each of the modules. In all of these systems, the focal point of the dendronpossessed either an azide or alkyne moiety. Wu and co-workers developed a 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) based asymmetric modular dendron with 16 mannose units and 2 coumarinchromophores, and demonstrated binding in a hemagglutination assay[61]. Goyal, Yoon, and Weck have also developed a poly(amine) dendrimer that possessed a single aldehyde or azide moiety on the dendrimer periphery capable of orthogonal functionalization by small molecule functional groups[62,63]. These approaches appear promising but only the bis-MPA dendron system has been demonstrated to have function as a targeted drug delivery platform. In all cases, the dendrons used were G3 or smaller. This has significant limitations for widespread therapeutic use because of the limited carrying capacity of low generation dendrons. To date, click chemistry has not been applied to a modular dendrimer system.

Considering the focus of this review article, the following sections provide a few examples of nanodelivery systems targeting cell organelles, specifically mitochondria and lipid bodies (LBs).

Mitochondria

Mitochondria, cellular power plants, play pivotal homeostatic role in cellular functions such as cellular signaling, growth and differentiation, cell cycle regulation, electron transport, calcium storage and cellular death[64,65]. Mitochondrial dysfunction is implicated in a variety of pathological disorders such as aging, ischemia-reperfusion, cardiac disorders, neurodegenerative and neuromuscular diseases, obesity, and genetic disorders[66–69]. One of the major causes of damage in these conditions is the generation of mitochondrial reactive oxygen species[70]. Some of the main disadvantages of selective drugs are their hydrophobicity, stability,bioavailability, inability to cross the membrane barriers and selective accumulation in the multi-membrane barrier organelles located in the cytoplasm, such as mitochondria. Targeting mitochondria with a variety of bioactive molecules and drugs is one strategy to overcome some of these hurdles[66]. Unlike cellular targeting, the prerequisite for mitochondrial targeting includes the use of drug modifications or encapsulation into nanocarriers such as dendrimers. This would help not only cross several membrane barriers, but also have high accumulation in these organelles. The other advantage of using the nanocarrier systems is their ability for site specific targeting with improved efficacy and reduced toxicity[71-74]. More

recently an interesting new approach was taken by Dhar's group[75]. This study showed the versatility of biodegradable high density lipoproteinnanoparticles for detection of plaques by targeting the collapse of the mitochondrial membrane potential. The same study described a rationally designed mitochondria-targeted polymeric nanoparticle (NP) system and its optimization for efficient delivery of various mitochondria-acting therapeutics by blending a targeted poly(d,l-lactic-co-glycolic acid)-block (PLGA-b)-poly(ethylene glycol) (PEG)-triphenylphosphonium (TPP) polymer (PLGA-b-PEG-TPP) with either nontargeted PLGA-b-PEG-OH or PLGA-COOH. An optimized formulation was identified through in vitro screening of a library of charge- and size-varied NPs. A programmable NP platform for the diagnosis and targeteddelivery of therapeutics for mitochondrial dysfunction-related diseases was also described[75]. The same group also showed how in situ light activation amplifies the host immune responses when NPs deliver the photosensitizer to the mitochondria, and opening up the possibility of using mitochondriatargeted-NP treated, light activated cancer cell supernatants as possible vaccines[76]. An overview of strategies to target organelles by exploiting different nanotechnological tools was recently reported[77].

Lipid bodies (LB)

Lipid bodies (LBs) are cytoplasmic organelles which have been historically considered cellular storage sites. LBs are phylogenetically conserved and ubiquitous organelles with many cellular functions[78-81]. More recently, they have been recognized as dynamic, communicating withdifferent organelles including mitochondria[82,83]. Different stressful conditions resulting inmitochondrial damage can lead to LB accumulation. The endoplasmic reticulum (ER) is a majorintracellular compartment involved in neutral lipid synthesis and LB biogenesis. Accumulation of LBs in leukocytes and macrophages follows their stimulation with pro-inflammatory agents including bacterial endotoxins (e.g., lipopolysaccharide from Gram negative bacteria) is well recognized[83,84]. Due to their prominence in inflammatory leukocytes, LBs are considered to be structural markers of inflammation. Therefore, pharmacological modulation of LB biosynthesis and composition presents an attractive strategy to correct LB abnormalities in different pathologies. To specifically target LBs, Kakkar and Maysinger developed a macromolecule-based delivery system using click chemistry[85]. The goal was to deliver niacin (and eventually other lipid-modifying drugs) to LBs by means of dendrimer and miktoarm polymer-based nanocarriers, in order to inhibit the activity of LB-localized enzymes.

An example of Diels-Alder "click" chemistry used for the delivery of drugs through peptides was reported by Braun's group[86]. It involved the delivery of a cytotoxic drug temozolomide (TMZ) using cyclic-RGD-ligand as cargo to target αvβ3 integrin receptor for cancer. The cytotoxic drug TMZ was ligated to the cRGD-ligand using Diels Alder reaction with inversion electron demand[86]. For evaluating the cellular location of this click product, a fluorescent tag dansyl was ligated. The cRGD-TMZ-dansyl complex when treated to MCF-7 cancer cells effectively binds on to the cell membrane which expresses high levels of αvβ3 integrin. This study also reports that the above click product selectively kills cancer cells with high efficacy as compared to only the TMZ drug treatment. In this section we provide examples of click chemistry to generate nanostructures targeting selected cellular organelles. Methodological details on imaging organelles have been recently reviewed[87].

Anticancer Drug Delivery

The high toxicity of conventional cytotoxic anti-cancer drugs often forces these agents to be given at sub-optimal dosages and this can result in treatment failure[88]. To resolve this problem, delivery platforms that can discriminate between healthy and malignant cells have been developed[88, 89]. Actively targeted therapeutic delivery platforms consist of three different components: a targeting component comprised of targeting ligands with affinities for molecules expressed on cancer cells; a payload consisting of drug and/or imaging agents; and a nano-scale structure to which the targeting and payload moieties are attached. This platform targeting of anti-cancer drugs with cancer cell-specific ligands can dramatically improve a drug's therapeutic index. Conjugating multiple targeting ligands to a single platform molecule further increases the potential for specific targeting of cancer cells by allowing the possibility of multivalent interactions[90, 91]. The structural design of these types of delivery platforms is critical to the success of the delivery device. Numerous classes of targeted drug delivery platforms have been developed that potentially meet the requirements needed to combine

targeting ligands, imaging agents, and drug molecules together to deliver the therapeutic payload to a desired location in the body. These include drug-target conjugates, linear polymers, lipid-based carriers (liposomes and micelles), carbon nanotubes, inorganic nanoparticles, and dendrimers. Several of these different delivery platforms are progressing towards or through clinical trials for cancer treatments with promising results[89]. Each approach, however, is not without limitations and the potential for widespread application of these platforms in their present design is unclear.

Dendrimer-based platforms have a unique branching structure which results in exceptionally high degrees of monodispersity and well defined terminal groups that provide the ability to form soluble conjugates containing multiple copies of hydrophobic drug and/or targeting molecules. The compact, branched structures appear to enhance the ability of the targeting molecules to interact in a fashion conducive to multivalent binding to cell membrane receptors[90]. The dendrimer's small size enables efficient diffusion across the vascular endothelium to find tumors and also allows the rapid clearance of these molecules from the blood stream. This clearance avoids potential long-term toxicities and reduces the necessity of a rapidly-degradable platform. The most widely used dendrimer in biomedical applications, poly(amidoamine) (PAMAM), is non-imunogenetic and non-toxic once the surface primary amines have been modified[92-97]. There have been numerous, recent examples describing the development of dendrimer-based targeted delivery systems using a wide variety of targeting ligands including monoclonal antibodies[98-102], peptides[103], T-antigens[104-106], and folic acid[107-116].

Despite the success of these dendrimer-based platforms, it has its own draw backs that include laborious chemical process, limited carrying capacity. These problems are sought by applying modular design concepts, where a dendrimer is used as module units, each with multiple copies of a single functional molecule. Multi-functional platforms can be generated by combining different modules through a universal coupling mechanism. Significant time is spent developing new orthogonal coupling strategies for desired functional combinations because many of the component drug molecules and targeting ligands (Taxol and RGD for example) are susceptible to a loss of activity due to undesired cross reactions as well as degradation by hydrolysis.

CONCLUSION

High fidelity coupling of alkynes with azidescatalyzed by copper has offered a useful platform in the tailoring and design of multifunctional nanocarriers, and in providing a detailed understanding of timely therapeutic interventions. Click chemistry has been utilized in developing a variety of multifunctional nanocarriers based on dendrimers and miktoarm polymers. These macromolecules, with their advantageous combination of properties, can bedirected towards specific cell organelles, including mitochondria and lipid droplets. Due to ease with which sequential “click” reactions can be performed in these macromolecules, this methodology can be extended to the design of novel nanocarriers with any desired combination of ingredients.The concept of dendrimers and dendritic structures containing an internal functionality is still a quite unexplored area. Within this concept, many new exciting materials and applications can be prepared.

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