J. Biol. Macromol., **23**(1), 13-17 (2023)

Review

The stability and reactivity of isothiocyanates and the plausible behavior of their dithiocarbamate- and thiourea-conjugates.

Toshiyuki Nakamura

Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan

Isothiocyanates (ITCs) are organosulfur compounds derived from cruciferous plants. An electrophilic ITC group (-N=C=S) reacts with some functional groups such as a hydroxyl ion, a thiol group and amine groups in proteins. It is known that ITCs are decomposed easily by the addition of a hydroxyl ion to the ITC group in aqueous solutions. In the intracellular behavior, the primary target of ITCs is a thiol group, which results in the formation of their dithiocarbamate conjugates. The reaction is considered to induce the health promoting and disease preventive effects of ITCs. ITCs also react with amine groups to form stable thioureas. This review offers a short summary of the stability and reactivity of ITCs and the plausible behavior of their dithiocarbamate- and thiourea-conjugates.

Received February 25, 2023; Accepted March 6, 2023

Key words: isothiocyanate; stability; modification; dithiocarbamate; thiourea.

Introduction

Isothiocyanates (ITCs) are one of the most famous organosulfur compounds having various biological activities. ITCs are enzymatic degradation products of glucosinolates (GLs), over 120 of which have been identified from various plants [1]. Among them, allyl ITC (AITC) is widely distributed as its GL, sinigrin, in cruciferous plants; sulforaphane (SFN) is abundant in broccoli in the form of glucoraphanin; phenethyl ITC (PEITC) is contained in watercress as gluconasturtiin; and benzyl ITC (BITC) is formed from glucotropaeolin in papaya (Fig. 1). ITCs are produced and then released when the plant tissue is

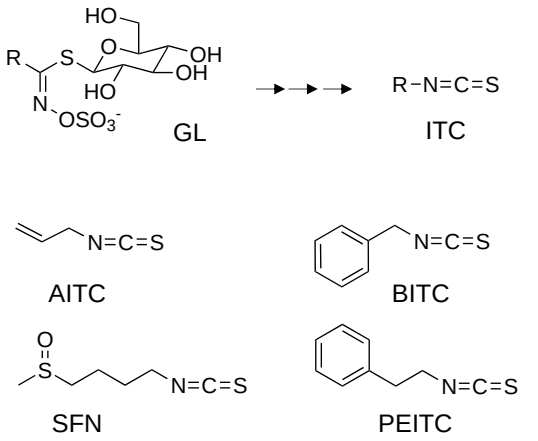

Fig. 1. Structure of GL and ITCs.
GL, glucosinolate; ITC, isothiocyanate; AITC, allyl ITC; BITC, benzyl ITC; SFN, sulforaphane; PEITC, phenethyl ITC.

E-mail: t-nakamura@okayama-u.ac.jp

damaged. The formation of ITCs is mediated by the hydrolysis of GLs by myrosinase in plants or myrosinase-like activity of the gut microflora in humans, followed by Lossen rearrangement under neutral conditions. ITCs are considered unstable in aqueous solutions because they are decomposed easily by the addition of a hydroxyl ion to the ITC group, although pure ITCs are relatively stable at neutral pH [2].

ITCs have been accepted as major ingredients that afford their health promoting and disease preventive potentials, such as anti-cancer, anti-inflammatory, and antioxidative effects [3]. As mention below, the chemical reactivity of ITCs might contribute to triggering their biological activities. The central carbon of ITCs is electrophilic and participates in their chemical reactions with nucleophiles. The primary nucleophilic target of ITCs in the cells is a thiol group of proteins and peptides. The reaction between ITCs and thiols results in the formation of dithiocarbamates (DTCs). The DTCs are unstable and readily undergo the reversed reaction to form free ITCs until equilibrium state. ITCs also react with amine moieties in proteins, such as the α-amino groups in the N-terminal residues, ε-amino groups in the lysine residues and secondary amine groups in the N-terminal proline residues. In contrast to the reaction of thiol groups, the thiourea conjugates with amine moieties are stable. This review summarizes the stability and reactivity of ITCs, and the possible involvement of DTCs and thioureas of ITCs in their health promoting effects.

Instability of ITCs

The instability of ITCs in aqueous solutions depends on the conditions, such as pH and temperature [2]. For example, ITCs in neutral or alkaline solutions are decomposed mainly by the addition of a hydroxyl ion to the ITC group and then form the thiocarbamic acids. In the thermal treatment (100 °C, 1 h), the remaining AITC was less than 30% in distilled water (adjusted to pH 7) [4]. On the other hand, the stability of BITC in water was found to be retained for 90 days at −20 °C and −80 °C, but not at 4 °C [5].

It has been shown that organic additives are potential solutions for improving the stability of ITCs in aqueous solutions. For example, the decomposition of AITC was retarded by inclusion complexation within the cavities of α-cyclodextrin (CD) and β-CD [6]. The decomposition of BITC and phenyl ITC was also suppressed by CDs, whose mechanism might be almost the same as that for AITC-α-CD complex [7]. In addition, the addition of L-cysteine (CYS) effectively prevented BITC from decomposition during 4 °C storage, and the amounts of BITC were maintained during storage for 14 days [5]. In contrast, *N*-acetylcysteine (NAC) or reduced form of glutathione (GSH) were ineffective in improving the BITC stability. Interestingly, CYS, but not NAC and GSH, easily conjugated with BITC to form the DTC conjugate, even though the free thiol groups of NAC and GSH as well as CYS were retained in the distilled water.

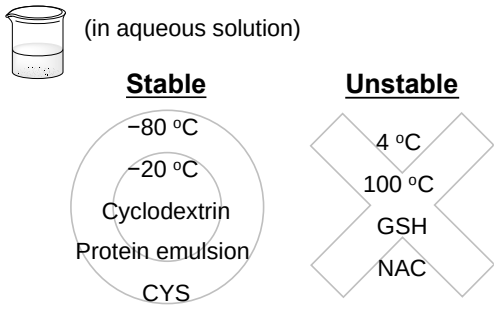

Fig. 2. Stability of ITC in aqueous solutions. CYS, cysteine; GSH, glutathione; NAC, *N*-acetylcysteine.

More recently, BITC can be embedded in protein emulsion systems, such as fish roe protein isolate emulsion and fish skin gelatin-sodium

alginate complex, to increase its stability and bioaccessibility [8,9]. Since the poor water solubility, low bioaccessibility, and basic unsteadiness of BITC limit the application of BITC in the food industry, the fish protein-based emulsion system is one of the promising strategies to protect and deliver unstable ITCs due to their non-toxicity, low cost and good accessibility. In any case, the decomposition of ITCs could be regulated by the frozen storage or certain organic additives in aqueous solutions (Fig. 2).

Intracellular behavior of ITCs

The intracellular behavior of ITCs *in vivo* is well understood. A primary target for ITCs in the cells is GSH, the most abundant cellular molecule with a thiol group. The reaction of ITCs with GSH results in the formation of DTCs, which is accelerated by glutathione *S*-transferases (GSTs). The GSH-conjugated DTCs are further converted into those of cysteinylglycine, cysteine and NAC. ITCs also bind to cysteine thiol groups in proteins directly or via thiol exchange reactions (Fig. 3).

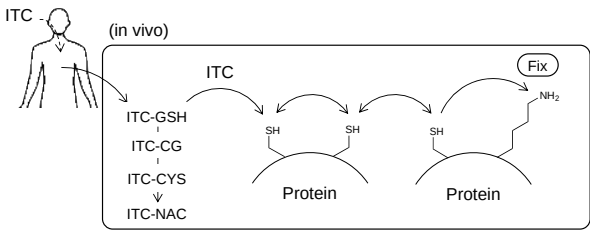

Fig. 3. Reactivity of ITC *in vivo*.
GSH, glutathione; CG, cysteinylglycine; CYS, cysteine; NAC, *N*-acetylcysteine.

The attack of ITCs on protein thiols are considered to, at least partly, initiate the health promoting effects of ITCs. Among the plausible target proteins for ITCs, Keap1, a cysteine-rich protein regulating the Nrf2-dependent gene expression and phenomena, has been most widely studied. In addition, the other proteins have been identified as ITC targets, such as tubulin, proteasome, and transient receptor potential channel A1 [2]. Although ITCs can also directly bind to DNA and RNA *in vitro*, their adducts of ITCs have not been detected in human lung cancer cells, suggesting that DNA and RNA are unlikely to be principal targets of ITCs in the cells [10].

Some reports have shown the biological activities of the ITC metabolites. The NAC conjugate of PEITC inhibited proliferation and tumorigenesis [11]. The cysteine conjugates of AITC and PEITC inhibited the growth and induced apoptosis in human leukemia cells [12]. In addition, the SFN metabolites have been reported to exhibit the similar cytotoxic and cytoprotective effects to those of SFN [13]. BITC–GSH conjugates also showed toxicity to the same extent as BITC, whereas BITC-NAC showed cytotoxicity less potently than BITC or BITC–GSH [14]. At a relatively higher concentration than BITC, the conjugates of BITC–GSH and BITC-NAC also induced DNA fragmentation in a nucleosome unit, which is the feature of apoptosis, and increased caspase-3 activity.

The intracellular accumulation of BITC is able to be quantified by the cyclocondensation assay that can detect both free BITC and its DTCs [15]. BITC was detected in the cells 30 min after treatment with BITC, then its intracellular level decreased gradually. The treatments of the BITC–GSH or BITC–NAC conjugates significantly increased the intracellular BITC accumulation, which was not so much as that by the BITC treatment. The increment of the intracellular BITC by the treatment of BITC–GSH conjugate was higher and faster than that of BITC–NAC conjugate. These results suggested that, compared with BITC–NAC, BITC–GSH was more unstable in aqueous solutions and more likely to occur the liberation of BITC from its metabolite.

Covalent modification of amine moieties

In addition to the DTC formation, ITCs can react with amine groups in proteins to form stable thioureas [2]. The reaction of ITCs and α-amino groups in proteins is well-known as Edman degradation, which is utilized for amino acid sequence analyses. In addition, the reaction of AITC and ε-amino groups of lysine residue under physiological conditions has been shown in the reaction mixture of albumin and AITC [16]. On the formation of thiourea, it has been considered that free thiol residues in a protein had no effect in the experiment using rabbit GAPDH. In fact, the ITC–lysine in albumin and hemoglobin have been identified in human plasma after ingestion of garden cress, watercress, and broccoli, even though thiol groups are abundant in the body [17]. In addition, BITC and PEITC are reported to inhibit irreversibly the tautomerase activity of macrophage migration inhibitory factor through covalent modification with the N-terminal proline residue [18-20].

A recent study has revealed the formation of BITC-lysine adduct in the cells in a time-dependent manner [14]. Furthermore, it has been detected that BITC covalently modified with caspase-3 by immunoprecipitation technique using an antibody against the BITC–lysine conjugate. The decreased activity of caspase-3 was consistent with the formation of BITC–lysine adducts, suggesting that BITC might inhibit the caspase-3 activity via covalent modification.

Regarding the reaction with lipids, the chemical reaction between ITC and an aminophospholipid, phosphatidylethanolamine (PE), has been reported [21]. The adduct of BITC and ethanolamine (EA) accumulated in a dose- and time-dependent manner in the medium when the cells were exposed to BITC. These results suggested that PE might be one of the primary targets of ITCs in vivo, and the BITC–PE adduct might be hydrolyzed by some types of phospholipases into BITC–EA adduct.

Conclusion

This review briefly summarized the stability and reactivity of ITCs. The stability of ITCs could be improved by the frozen storage or certain organic additives, although ITCs in an aqueous solution are quite unstable. In vivo, ITCs react primarily with thiol groups to form DTCs. It has been considered that the DTCs as well as ITCs themselves have the potentials to show health promoting and disease preventive effects. In addition, ITCs react with amine moieties to form the stable thiourea conjugates. The reaction of ITCs and amine moieties has not been adequately explored because the rate of the reaction is much less than the DTC formation. However, the thiourea formation might not only be a useful marker, but also contribute to beneficial health effects of ITCs. Future works will be concerned with the functionality of the thioureas of ITCs as well as DTCs.

References

1. Fahey JW, Zalcmann AT, Talalay P. *Phytochemistry*. 2001, **56**, 5–51.
2. Nakamura T, Abe-Kanoh N, Nakamura Y. *J Clin Biochem Nutr*. 2018, **62**, 11-19.
3. Dinkova-Kostova AT, Kostov RV. *Trends Mol Med*. 2012, **18**, 337-347.
4. Chen CW, Ho CT. *J Agric Food Chem*. 1998, **46**, 220-223.
5. Nakamura T, Murata Y, Nakamura Y. *Food Chem*. 2019, **299**, 125118.
6. Ohta Y, Takatani K, Kawakishi S. *Biosci Biotechnol Biochem*. 2000, **64**, 190-193.
7. Ohta Y, Matsui Y, Osawa T, Kawakishi S. *Biosci Biotechnol Biochem*. 2004, **68**, 671-675.
8. Tang Y, Wang X, Jiang H, Song L, Cui H, Zhang Z, Lin S. *J Food Sci*. 2020, **85**, 2923-2932.

9. Zhang X, Liu Z, Wang A, Zhang S, Nakamura Y, Lin S, Tang Y. *J Sci Food Agric*. 2022, **102**, 5680-5689.
10. Mi L, Wang X, Govind S, Hood BL, Veenstra TD, Conrads TP, Saha DT, Goldman R, Chung FL. *Cancer Res*. 2007, **67**, 6409–6416.
11. Chiao JW, Wu H, Ramaswamy G, Conaway CC, Chung FL, Wang L, Liu D. *Carcinogenesis*. 2004, **25**, 1403-1408.
12. Xu K, Thornalley PJ. *Biochem Pharmacol*. 2000, **60**, 221-231.
13. Liu P, Wang W, Zhou Z, Smith AJO, Bowater RP, Wormstone IM, Chen Y, Bao Y. *Nutrients*. 2018, **10**, 585.
14. Nakamura T, Tsutsui C, Okuda Y, Abe-Kanoh N, Okazawa S, Munemasa S, Murata Y, Kato Y, Nakamura Y. *J Biochem Mol Toxicol*. 2022, **36**, e23184.
15. Zhang Y, Wade KL, Prestera T, Talalay P. *Anal Biochem*. 1996, **239**, 160-167.
16. Nakamura T, Kawai Y, Kitamoto N, Osawa T, Kato Y. *Chem Res Toxicol*. 2009, **22**, 536-542.
17. Kumar A, Sabbioni G. *Chem Res Toxicol*. 2010, **23**, 756-765.
18. Cross JV, Rady JM, Foss FW, Lyons CE, Macdonald TL, Templeton DJ. *Biochem J*. 2009, **423**, 315–321.
19. Brown KK, Blaikie FH, Smith RA, Tyndall JD, Lue H, Bernhagen J, Winterbourn CC, Hampton MB. *J Biol Chem*. 2009, **284**, 32425–32433.
20. Ouertatani-Sakouhi H, El-Turk F, Fauvet B, Roger T, Le Roy D, Karpinar DP, Leng L, Bucala R, Zweckstetter M, Calandra T, Lashuel HA. *Biochemistry*. 2009, **48**, 9858–9870.
21. Nakamura T, Hirakawa M, Nakamura Y, Ishisaka A, Kitamoto N, Murakami A, Kato Y. *Chem Res Toxicol*. 2019, **32**, 638-644.

Communicated by Kiyoshi Yasukawa