Adsorptive Interactions between Carbon Nanotubes and Complex Cell Culture Media

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INTRODUCTION

Nanomaterial / cell interactions are fundamental to both the toxicity profiles and biomedical applications of new nanomaterials (R. H. Hurt et al, 2006, Smart et al. 2006, Colvin, 2003). In addition to direct interactions (endocytosis, phagocytosis, membrane attachment), nanomaterials may influence cell behavior indirectly by adsorbing, deactivating, or destroying biological solutes in extracellular fluid or cell culture medium (Monteiro-Riviere et al, 2006, Casey et al. 2007).Previous research showed single wall carbon nanotubes (SWNT) can interact with a wide arrange of biological probes and fluorescent dys. (Worle et al. 2006, Casey et al, 2007). Some cell culture media components, like cysteine, are reducing agents that could participate in chemical reactions with oxygen functional groups on carbon surfaces or metal oxide residues in SWNT. Biological solutes may be attacked by free radicals produced by Fe catalyzed redox cycling (Smith K. R. et al, 1997). Here we consider the alteration of cell culture medium by single-wall carbon nanotubes, whose high surface area and significant hydrophobicity make them powerful sorbents for many biological solutes. In this paper, we will present new experimental results on the interaction of single-wall nanotubes with media, including amino acid and vitamin profiles, and quantitative adsorption isotherms of biological molecular probes, for both as-produced and functionalized nanotubes.

MATERIALS AND METHODS

Materials: SWNT were purchased from Carbon Nanotechnologies, Inc, which were made by the HipCO method and purified by the vendor. There is 10.9% Fe as determined by ICP. Phenol red and L-tyrosine were purchased from Fisher Scientific Inc. The chosen cell culture media were RPMI Media 1640-11835 (-phenol red) and 1640-11875(+phenol red) from Invitrogen Corporation. Phosphate-buffered saline (PBS) buffer was diluted ten times from10X PBS form EMD, pH was adjusted to pH 7.4.

Adsorption isotherm: The following experiments were carried out to determine the adsorption isotherm of phenol red and tyrosine. Phenol red and tyrosine solutions were prepared in PBS buffers. SWNTs were dispersed into those phenol red/tyrosine solutions and sonicated for half an hour. The solutions were then incubated at room temperature on a rotation motor for 24 hours, which was kept rotating for 360^{o}. Solutions were transferred to 4 ml centrifugation tubes containing integrated filters and centrifuged at 4400 rpm at 4 ^{o}C. Supernatants of phenol red were tested by spectrophotometer with model number: SPECTRAmax M2 at 560 nm. Supernatants of tyrosine were tested at 280 nm.

Desorption isotherm: The following experiment was carried out to determine the desorption isotherm of phenol red and tyrosine. A series of SWNTs were dissolved into PBS buffer at the same concentration and sonicated for half an hour. Then they were incubated for 24 hours at the same condition as adsorption isotherm. Then samples were diluted to different volumes and incubated for another 24 hours. At last the solutions were transferred to centrifugation tubes with integrated filters and centrifuged at 4400 rpm at 4 ^{o}C. Testing methods are as same as adsorption isotherm.

Phenol red adsorption isotherm in cell culture media: Phenol red was added to RPMI 1640-11835 cell culture media, which was originally free of phenol red. This solution was used to measure the CNT adsorption isotherms as described above.

Functionizaation of SWNTs: Sulfanilic acid aqueous solution was made by deionized water with the weight ratio of acid to water: 0.7:480. Then SWNT were dispersed into the acid solution with concentration 1 mg/ml. The solution was sonicated for 1 hour. Then solution was placed in 70 ^{o}C water bath and then $NaNO_2$ aqueous solution (weight ratio of salt to water is 0.28:20) was added drop by drop. The weight ratio of total added salt solution to acid solution was 24:1. The reaction mixture was held for one more hour at 70 ^{o}C under stirring. The mixture was then transferred to a refrigerator to quench the sulfonation. The mixture solution was centrifuged at 4400 rpm and deionized water was used to wash the product 5 times. Finally the product was dried at 100 ^{o}C.

Amino acid and vitamin profiling: Different doses of SWNT were dissolved into RPMI 1640-11875 cell culture media and sonicated for half an hour. Then samples were cooled down to -10 ^{o}C and sent to Sigma-Aldrich for "spent media analysis". Amino acids were analyzed by HPLC using UV and fluorescence detection. Water-Soluble vitamins were analyzed by HPLC (Agilent 1100 CapLC) using MS-MS detection (Thermo Electron LTQ).

Zeta potential: SWNTs were dispersed into deionized water and sonicated for an hour. The solutions were then centrifuged for one minute to remove larger aggregates. The gray supernatant was transferred to vials and the Zeta potential at room temperature measured by Zetasier Nano-zs instrument (forom Malvern Instruments).

RESULTS AND DISCUSSION

Before looking at the adsorptive behavior of specific solutes in cell culture medium, we can begin by examining the basic stoichiometric relation between dose, nanomaterial surface area, and concentration change that govern the behavior of any nanomaterial/solute system. Figure 1 examines the implications of single monolayer coverage in limit of high adsorption potential. The surface area of SWNT can reach or exceed 500 m^2/g. From Fig. 1, we can see that at this surface area, the potential maximum concentration change can be tens of ug/ml at dose of 100 ug/ml. In cell culture media, the concentrations of most amino acids are lower than 100 ug/ml. And those of most vitamins are as small as 1 ug/ml. The interaction between these components in cell culture media and SWNTs must therefore be considered.

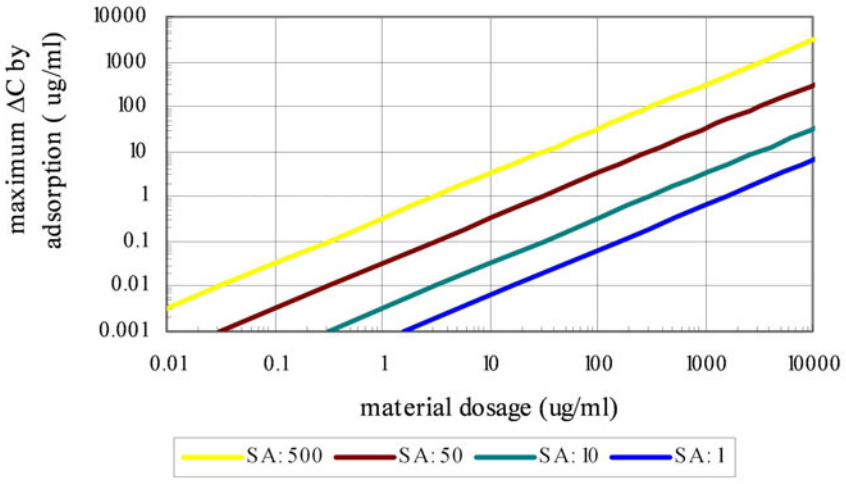

Fig. 1: Potential for nanomaterials to alter biological fluid phases by adsorption. Plot gives maximum concentration change by adsorption at monolayer coverage for materials with various surface area (SA: m^2/g) and dose. This example calculation is for typical of small (non-macromolecular) biomolecular solutes, assuming 1nm^2 surface area/ molecular and only monolayer physical adsorption

For this study, we focus on RPMI 1640 cell culture media interacting with various doses of SWNT (from 0.01 mg/ml to 10 mg/ml). The concentration changes of amino acids are shown in Fig. 2. The interaction levels of the amino acids differ greatly; some are essentially unchanged, while others drop in concentration by up to 90%. For analysis purposes we separate them into three groups by the fractional concentration change at a dose of 10 mg/ml. Those with concentrations changes less than 10% are presented in Fig. 2 a). Those amino acids are: Asp, Glu, Ser, Thr, Lys and Gly. The concentrations change between 10 % and 50% are presented in Fig. 2 b). The amino acids in this region are Leu, Ile, Hyp, Gln, Asn and Val. The concentrations change larger than 50% are presented in Fig. 2 c). The amino acids in this region are His, Cys, Met, Arg, Tyr and Phe. We observe that most of amino acids with the largest concentration changes in the presence of SWNT have side chains classified as hydrophobic and those with little concentration change have side chains classified as hydrophilic. This implies that the material's hydrophobicity play an important role in the interaction with SWNT.

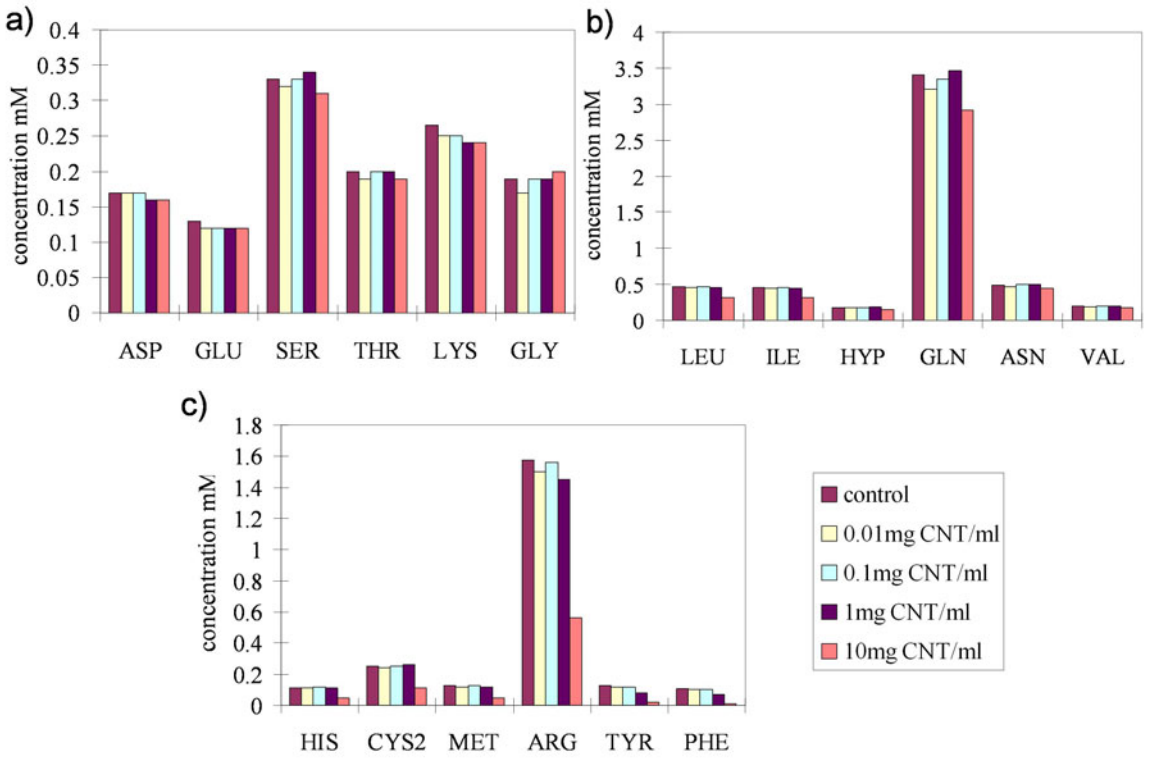

Fig. 2: a) CNT adsorption of amino acids in cell culture media ($\Delta C < 10\%$ by 10 mg/ml SWNT); b) CNT adsorption to amino acids of cell culture media ($10\% < \Delta C < 50\%$ by 10 mg/ml SWNT); c) CNT adsorption to amino acids in cell culture media ($\Delta C > 50\%$ by 10 mg/ml SWNT).

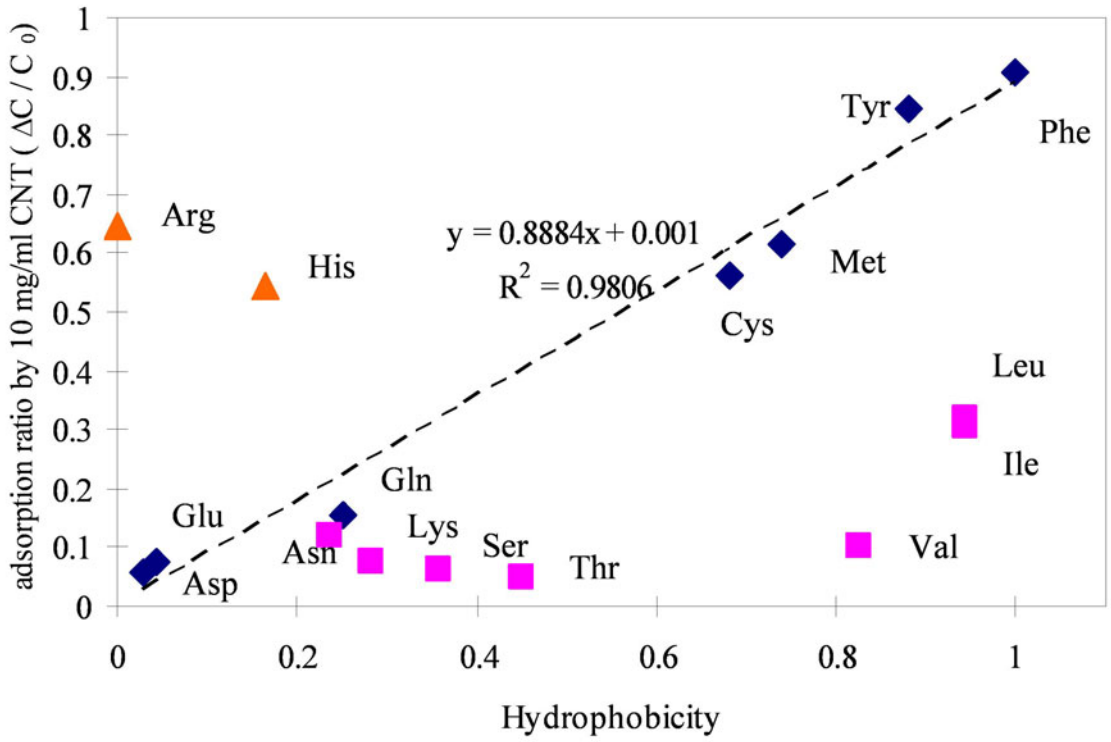

Fig. 3 Correlation of fractional amino acid adsorption at 10 mg/ml SWNT with hydrophobicity index.

In Fig. 3, we compare the maximum concentration change of the amino acids with their hydrophobicity index. Some points showed linear relationship between their concentration change and hydrophobicity. From Fig. 3, Arg and His are significant outliers. which are hydrophilic but have high concentration change by SWNT. The unusually strong adsorption of Arg and His may be partly due to their basic side chains. The zeta potential of SWNT is – 26. mV, which indicates that SWNTs have a net negative surface charge at the neutral pH of these experiments. Electrostatic attraction may aid in the adsorption. These groups have been observed to show strong physical interactions with carbon surfaces. The amino acids that lie far below the linear trendline are branched-chain aliphatics with three-dimensional structure, which are not capable of pi-bonding and likely to be more weakly adsorbed than planar or near-planar aromatic molecules.

Fig. 4 presents the concentration changes of vitamins in cell culture media as a function of SWNT dose. The data shows that vitamins are effectively depleted at low SWNT dose. Thiamine, folic acid and riboflavin were below the detection limit after exposure to only 0.1 mg/ml SWNT. Comparing the chemical structure between these three vitamins with others in Fig. 4, we note the presence of multi-ring aromatic structures and ring-bound nitrogen. Previous research from showed that SWNT are a good sorbent for conjugated polymers and carbon surfaces are known to have a high affinity for planar polyaromatic compounds (Hurt et al. 2002, Radovic), and some N-containing solvents.

To better understand the interactions of SWNT and cell culture media and its mechanisms, we measure quantitative adsorption isotherms for phenol red, a pH indicator, by SWNT, as presented in Fig. 5. The orange dots in Fig. 5 present results for the functionalized SWNT. The original SWNT were functionalized by aryl-sulfonation and become more hydrophilic with a Zeta potential of -54.9 mV compared to the original SWNT with zeta potential of – 26.4 mV.

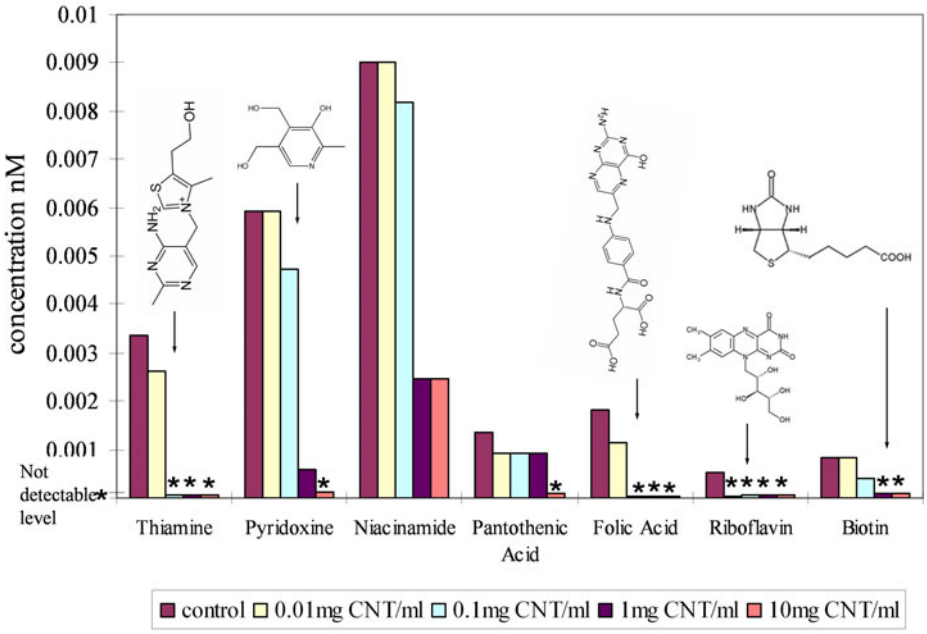

Fig. 4: Dose-dependent CNT adsorption of vitamins from cell culture media, * shows not detectable levels.

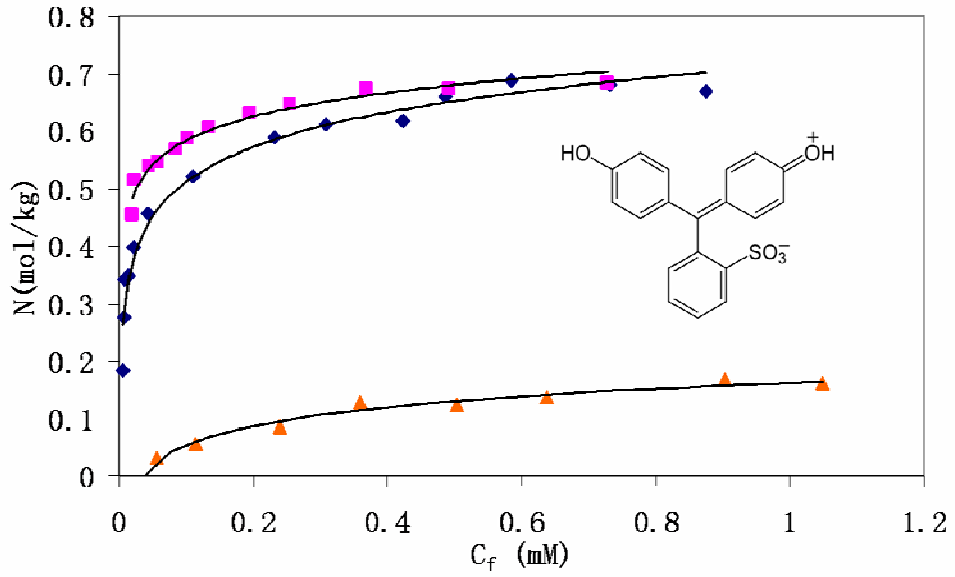

Fig. 5: Adsorption isotherm for phenol red on untreated SWNTs with chemical structure of phenol red: purple squares give the desorption isotherm for untreated SWNT, blue diamonds give the adsorption isotherm for untreated SWNTs, and orange triangles give the adsorption isotherm for aryl-sulfonate functionized SWNT

Fig. 6 shows the adsorption and desorption isotherm of tyrosine, an essential amino acid. The adsorption is not fully reversible, indicating either chemisorption on selected surface sites or strong physical adsorption in or this aromatic compound that prevents full desorption over the course of the experiment.

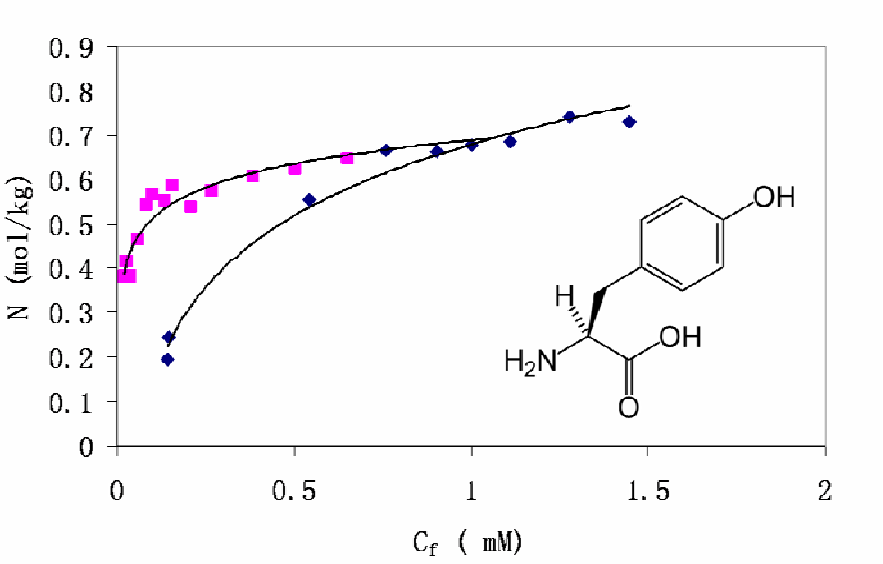

Fig. 6 Adsorption and desorption isotherms for tyrosine on by SWNTs. Purple squares shows desorption isotherm; blue diamonds shows adsorption isotherm.

Figure 7 compares the adsorption behavior of phenol red from cell culture medium with its adsorption behavior from simple buffer solutions. The lesser extent of adsorption from medium is believed to reflect the highly multicomponent nature of medium in which over 40 components must compete for carbon adsorption sites.

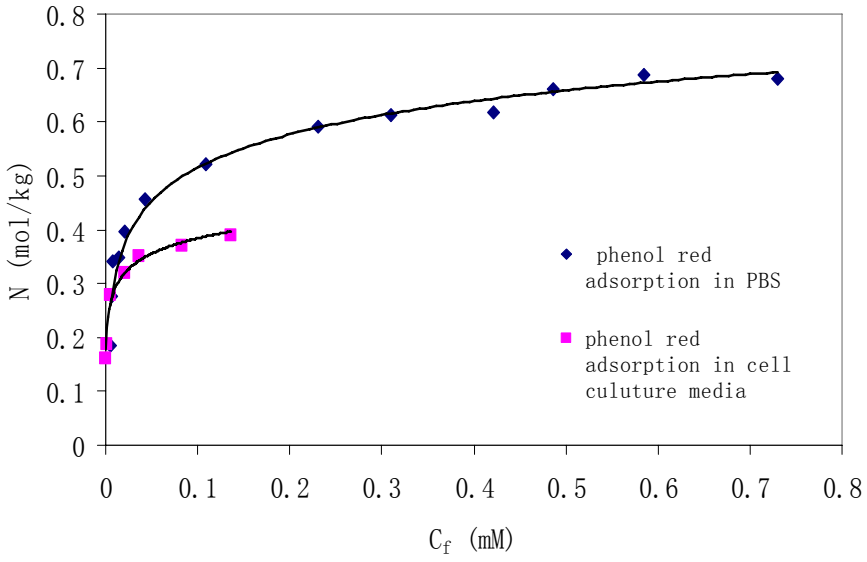

Fig. 7: Adsorption isotherm of phenol red and tyrosine in cell culture media and comparison with the isotherm in simple buffers: purple squares shows desorption isotherm in cell culture media; blue diamonds shows adsorption isotherm in PBS buffer solution.

CONCLUSIONS

Overall we have shown that single-wall carbon nanotubes in cell culture medium show significant adsorption of a variety of small molecule solutes, including amino acids, vitamins, and the pH indicator dye, phenol red. These quantitative measurements show significant depletion for SWNT doses as low as 0.01 mg/ml, and suggest that small molecule adsorption will be an important issue in the study of nanotube interactions with physiological fluid phases and fluorescent or colorimetric molecular probes used in nanotoxicology and nanomedicine.

ACKNOWLEDGEMENT

Financial support was provided by EPA STAR Grant RD-83171901-0, the National Science Foundation, DMI-0506661, and the SBRP Program of the NIEHS.

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