Raman microspectroscopic study of biomolecular structure inside living adhesive cells

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Abstract Cells adhesion is very important for many physiological processes. Using advanced Raman microspectroscopic technique, we selected T Leukemia cells (Jurkat) as the materials and obtained simultaneously conformation information of various biomolecules inside the whole living cells. By comparing the Raman microspectroscopic spectra of single and adhesive cancer cells, we found for the first time that when cells adhered, the conformation of the biomolecules (DNA, protein, carbohydrates and lipids) inside the cells had different changes: (i) the backbone of double-stranded DNA maintained orderly B-form or modified B-form conformation, whereas the groups of its deoxyribose and bases were modified; (ii) the conformational changes of the main chain and the side chain in the protein were obviously variant. The lines intensity belonging to α-helix and β-sheet decreased, while that of β-turn increased. Tyrosine and tryptophane residues of the protein changed from "buried state" to "exposed state"; the lines intensity of its sulfhydryl group also increased; the conformation of its disulfide bond changed from two kinds to three kinds. These facts suggest that the cells adhesion causes changes in H-bonds organization of the main chain and environment of the side chain in the protein; (iii) the groups of the carbohydrates were also modified simultaneously; (iv) the conformation of the lipids bilayers of the membranes changed obviously; the order parameter for lateral interaction between chains decreased gradually with the increase of number of the adhesive cells. So cells adhesion resulted in an increase in fluidity of the membrane and ion permeability on the membrane.

Keywords: Raman microspectroscopy, living cells, biomolecular structure, cell adhesion.

Cells adhesion is very important for many physiological processes such as morphogensis, immune response, wound closure, and the metastasis of cancer as well[1—3]. Adhesion junctions are formed by transmembrane adhesive proteins linked to cytoskeletal network[4,5]. These junction structures maintain and promote homotypic cells adhesion. In addition, increasing amounts of evidence are accumulated to suggest that cells adhesion can transfer intercellular signals and regulate cellular physiological activities[4,6—8].

The influence of cells adhesion on the function of cells has been studied[9,10]. For example, Zhuang proved that if undifferentiative lung bud epithelia was cultured together with interstitial cells from different sources, they would develop into different tissues. But the lung bud epithelia cultured singly would not differentiate into different tissues[10]. Gurdon also proved that mesoblast cells induced could not differentiate unless they were under the aggregate state. He called this

phenomenon "Community Effect"[9]. These results suggested that cell adhesion might affect and even control the differentiation of embryonic cells. In the invasiveness processes of cancer cells, cancer cells may invade walls of vessels and move into surrounding tissues, then they adhere to normal cells and make these normal cells cancerate[1]. It is probable that the cancer cells affect the intercellular communication of the normal cells they adhere, making the cells cancerate[1,11].

At present, the mechanisms and processes of cell adhesion are studied extensively[5,12—14], but what change occurs in the structure of biomolecules (DNA, protein, carbohydrate and lipids) inside adhesive cells? This important problem remains unsolved. One of the reasons is due to lacking of satisfactory and direct methods to detect the influence of cells adhesion on biomolecular structures inside the cells.

In recent years, the Raman microspectroscopy technique which provides Raman spectra and bright field microscopic images of cells has been developed for studying whole living cells[15]. Raman spectra could provide simultaneously direct evidence for changes of various biomolecular structures inside the whole living cells, involving conformation of phosphate backbone, deoxyribose and four bases of DNA, main and side chains of protein, as well as lipids and carbohydrate.

In this work, by comparing the Raman spectra of single and adhesive living cells, we analyzed the changes in conformation of biomolecules (DNA, protein, lipids, and carbohydrate) inside adhesive cancer cells, and found that the conformation of biomolecules inside the cells had obviously different changes with the increase of number of adhesive cells. We believe that these new results will help to explain the influence of cells adhesion on the function of cells at the molecular level.

1 Materials and methods

1.1 Materials

Jurkat T Leukemia cells were obtained from Beijing Medical University. The cells were cultured in RPMI 1640 (Sigma Chemical Co., St. Lunis, MO) supplemented with 15% heat-inactivated fetal bovine serum (Sigma Chemical Co.), at 37℃ in a 5% CO_2 atmosphere.

The Jurkat cells were centrifuged (70×*g*) and washed five times with PBS (pH 7.0). Then the cells in PBS were deposited onto a glass-slide and covered with a cover-glass.

1.2 Spectral measurements

Each Raman spectrum and bright field microscopic image were obtained on a France Jobin Yvon S.A T64000 Raman spectrograph with a triple monochromator, which was fitted with a detector liquid-nitrogen-cooled charge-coupled-device (CCD), a microprobe ×50 Olympus BX40 objective (NA 0.75), computer and an Argon-ion laser (model 169 Spectra-Physics. U.S.A.). The experimental conditions were as follows: exciting line 514. 5 nm; power 6 mW; focus on the nucleus; measuring time 300 s; scanning ranges: (1) 400—1800 cm^{-1}, (2) 2500—3100 cm^{-1}; widths of slits 500 μm; typical resolution 0.5 cm^{-1}; and room temperature (20±2℃). Raman signals of

PBS buffer were subtracted.

2 Results and discussion

The Raman spectra of a single and adhesive cancer cells in different spectral regions are shown in figs. 1—3. The bright field microscopic images of single and adhesive living cells are shown in fig. 4, which indicates the state of adhesive cells detected. The Raman lines in figs.1—3 come from the contributions of four major components of the cells —nucleic acid, protein, lipids and carbohydrate. For narrating simply and conveniently, we use "single, two, three, and a group" to represent the single, two, three and a group adhesive cells, respectively. According to the methods and data of Thomas, Lord, Carey, Tu and Gaber et al.[16—32], their Raman spectroscopic characteristics are interpreted respectively as follows:

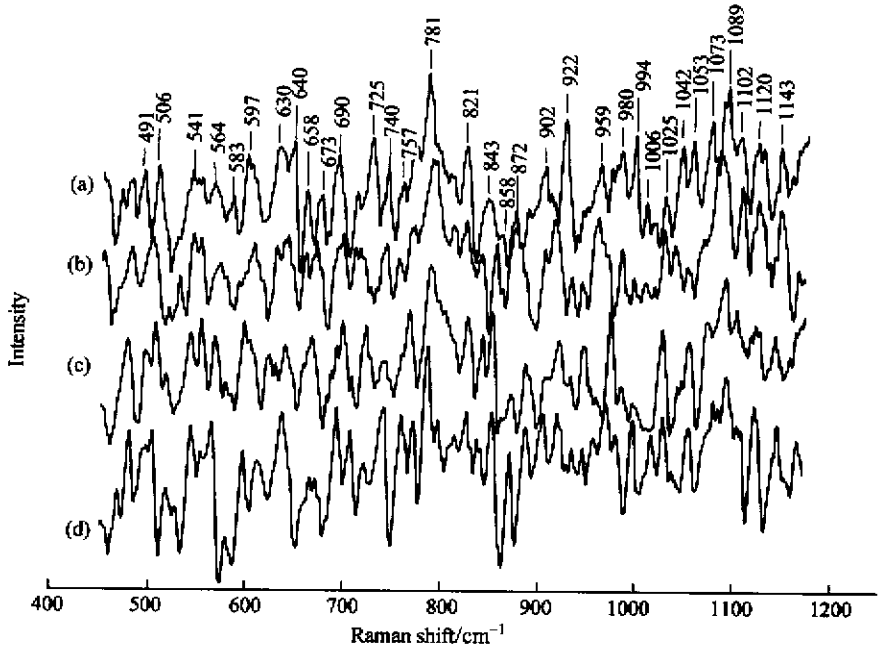

Fig. 1. Raman spectra of interactive T Leuk(a)emia cells (Jurkat) (450—1170 cm^{-1}). (a) A single cell; (b) two adhesive cells; (c) three adhesive cells; (d) a group of adhesive cells. Experiment conditions were as follows: Exciting line 514.5 nm; power 6 mW; focus on the nucleus; measuring time 300 s; scanning ranges: (1) 400—1800 cm^{-1}, (2) 2500—3100 cm^{-1}; widths of slits 500 μm; typical resolution 0.5 cm^{-1} and room temperature was 20±2℃. Raman signals of PBS buffer were subtracted.

2.1 The conformation of DNA inside single and adhesive living cancer cells

The lines of DNA were very projecting in the Raman spectra of the cells. The lines assigned to the backbone po_2^- group symmetrically stretching occurred at 1089 (single), 1084 (two), 1089 (three) and 1091 cm^{-1} (a group)[15], and the lines assigned to backbone po_2 phosphate diester symmetrically stretching appeared at 781 (single), 786 (two), 785 (three) and 784 cm^{-1} (a group)[15], respectively. While the lines of B-form or modified B-form conformation occurred at 843 (single),

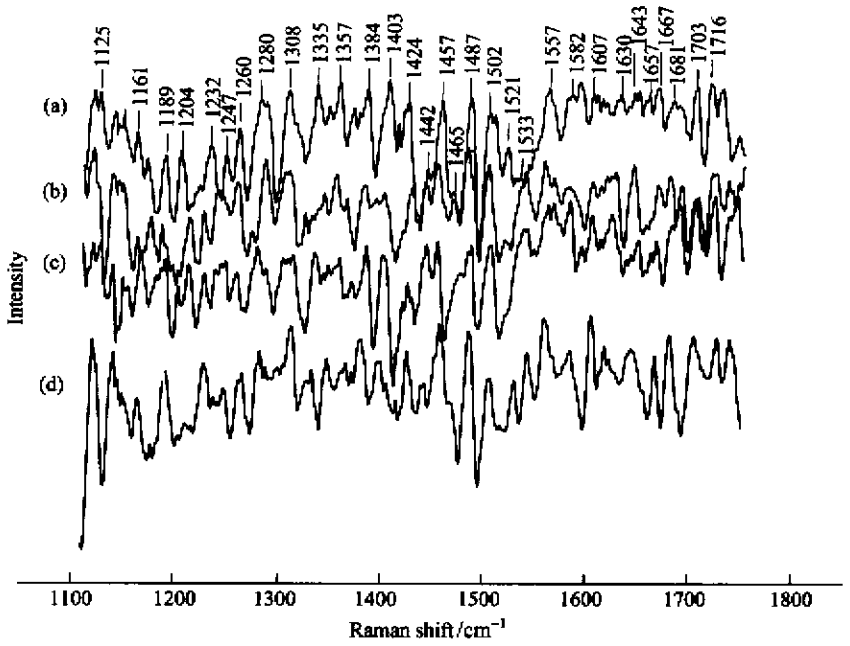

Fig. 2. Raman spectra of interactive T Leuk(a)emia cells (Jurkat) (1110—1750 cm^{-1}). Explanation as in fig. 1.

838 (two), 840 (three) and 835 cm^{-1} (a group)[16,17] respectively, in Raman spectra of the single and adhesive cells. It is indicated that the backbone structure of the double-stranded DNA remained orderly. Whereas the bands assigned to the groups of deoxyribose at 922, 980, 1053, 1143, 1442 and 1465 cm^{-1}[15,17,19] changed clearly, the intensities of the two lines assigned to deoxyribose at 1442 and 1465 cm^{-1} increased. The line belonging to deoxyribose at 922 cm^{-1} shifted to 912 cm^{-1} (two), 918 cm^{-1} (three) and 917 cm^{-1} (a group), and its intensity decreased with the increase of number of adhesive cells. The bands belonging to various groups of bases —adenine (A), guanine (G), cytosine (C) and thymine (T) at 673(T), 725(A), 757(T), 1260(C, A), 1308(A), 1335(A), 1384(T, A, G), 1424(A, G), 1487(G, A), 1502(A), 1521(A), 1533(G, C), 1582(G, A) and 1667(T, C=O) cm^{-1}[15,17,18] had respective changes. A number of the bands shifted and their intensities decreased or increased, for instance, the intensity of the line assigned to cytosine and adenine at 1260 cm^{-1} decreased and it shifted to 1265 cm^{-1} (a group); the intensities of the two lines belonging to adenine, and adenine, guanine at 1335 cm^{-1} and 1424 cm^{-1} also decreased respectively and the former shifted to 1325 cm^{-1} (two), 1334 cm^{-1} (three) and 1332 cm^{-1} (a group); the intensity of the two lines for guanine, cytosine at 1533 cm^{-1}, and guanine, adenine at 1582 cm^{-1}, increased with the increase of number of the adhesive cells. The former shifted to 1538 cm^{-1} (two), 1540 cm^{-1} (three), and 1541 cm^{-1} (a group), respectively, while the latter shifted somewhat. It suggests that the groups of deoxyribose and bases had been modified[17,18]. Nearly every modification of the components of DNA can lead to changes in metabolism and genetic quality of cells.

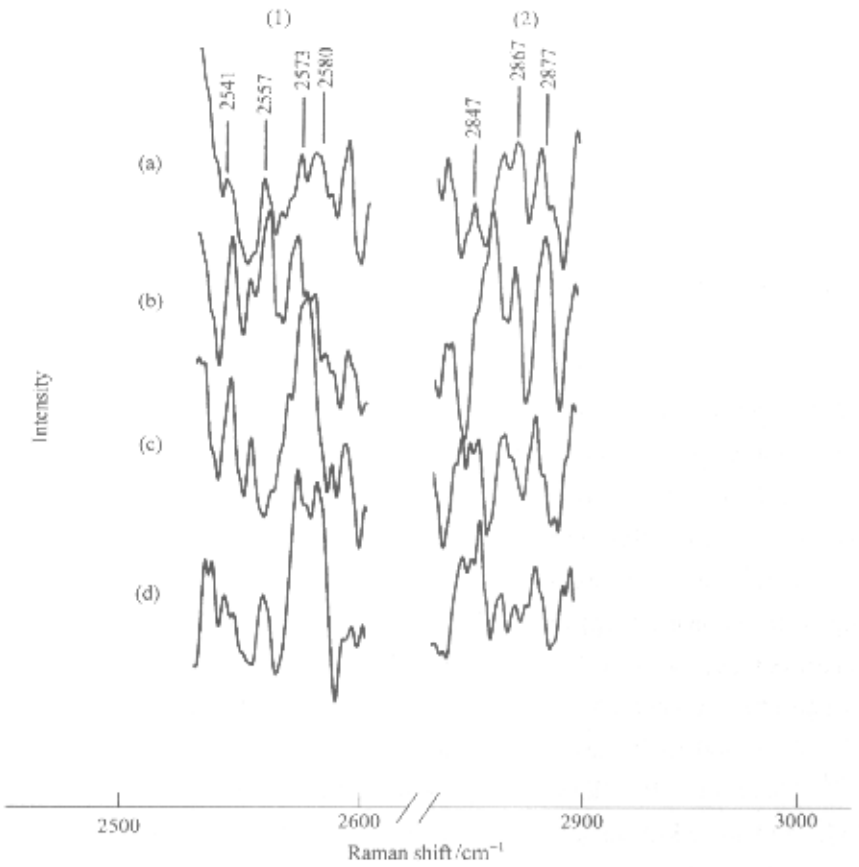

Fig. 3. Raman spectra of interactive T Leuk(a)emia cells (Jurkat). (1) Sulfhydryl group of protein; (2) CH_2 group of lipids. Explanation as in fig. 1.

2.2 The conformation of the protein inside single and adhesive living cancer cells

A majority of cells are consisted of proteins. The change of proteins is one of the important reasons why cellular functions change. Various changes of the conformation-sensitive lines in the proteins of adhesive cancer cells are stated as follows:

(1) Main-chain conformation: The line of α-helix at 1280 cm^{-1} (single)[19,20] shifted to 1285 cm^{-1} (two), 1278 cm^{-1} (three) and 1281 cm^{-1} (a

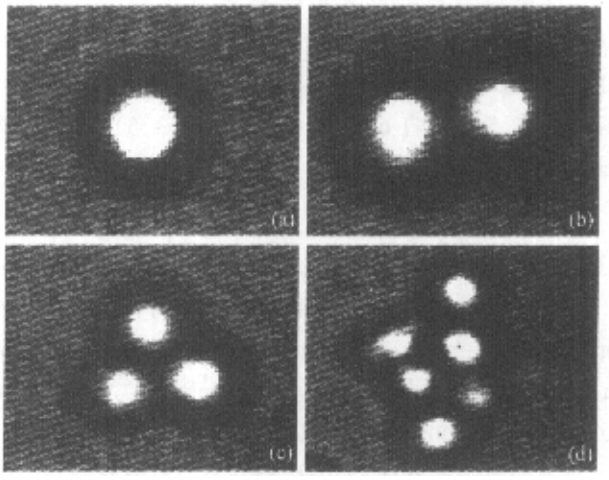

Fig. 4. The bright field microscopic images of living T Leuk(a)emia cells (Jurkat). (a) A single cell; (b) two adhesive cells; (c) three adhesive cells; (d) a group of adhesive cells. ×50 Olympus BX40 objective (NA 0.75); focus on the nucleus.

group), while the line assigned to α-helix at 1657 cm^{-1} (single)[19,20] shifted to 1656 cm^{-1} (two), 1657 cm^{-1} (three) and 1654 cm^{-1} (a group), respectively, and their intensity decreased gradually. The line belonging to β-sheet at 1232 cm^{-1}[19,20] (single) shifted to 1239 cm^{-1} (two), 1233 cm^{-1} (three) and 1236 cm^{-1} (a group), respectively, and their intensity decreased obviously. The line assigned to β-turn at 1308 cm^{-1} (single and two)[21,22] shifted to 1312 cm^{-1} (three and a group), respectively, while the line at 1681 cm^{-1} (single)[22,23] shifted to 1690 cm^{-1} (two), 1688 cm^{-1} (three) and 1680 cm^{-1} (a group), their lines intensity increased with the increase of number of adhesive cells.

(2) Side-chain conformation: The intensity ratios (I_{850}/I_{830}) of the double peaks of tyrosine (Tyr) residue which arose from the Fermi resonance between a ring-breathing vibration and the overtone of an out-of-plane ring bending vibration which occurred at 858, 821 cm^{-1} (single), 854, 822 cm^{-1} (two), 850, 824 cm^{-1} (three) and 851, 824 cm^{-1} (a group), were 0.33 (single), showing that the Tyr was "buried". But the ratio varied from 1.06 (two) to 1.22 (three) and 1.23 (a group) when the cells adhered, denoting that the Tyr was "exposed"[19,20]. The ten lines assigned to the indole-ring of tryptophane (Trp) changed to certain degrees, in which the line at 1357 cm^{-1} remained sharp (single, two and three), showing that Trp was "buried"[19,20], whereas it became a shoulder (a group), showing that Trp was "exposed". The line belonging to phenylalanine (Phe) at 1006 cm^{-1} shifted and its intensity decreased gradually, which almost disappeared (three and a group)[19,20]. There were four lines belonging to sulfhydryl group (-SH) stretching vibration at 2541, 2557, 2573 and 2580 cm^{-1}, in which the intensities of the three lines at 2541, 2573 and 2580 cm^{-1} increased with the increase of number of adhesive cells. It suggests that some enzymes inside the adhesive cells have been activated[24]. There were two kinds of conformation of disulfide bond —gauche-gauche-gauche (g-g-g) and trans-gauche-trans (t-g-t) in the single cell, which appeared at 506 and 541 cm^{-1}, respectively, whereas there were three kinds of conformation of disulfide bond (g-g-g, g-g-t, t-g-t) in the adhesive cells, which occurred at 501, 520, 529, 543 cm^{-1} (two), 505, 520, 541 cm^{-1} (three) and 503, 518, 528, 542 cm^{-1} (a group), respectively. It suggests that cells adhesion results in changes in H-bonds organization of the main chain and environment of the side chain in protein.

2.3 The conformation of carbohydrate inside single and adhesive living cancer cells

The glycosyl- of biomolecule not only plays an important role in carrying and delivering information, but also has bioactivity in regulating metabolism. Participating in cellular adhesion is an important function of glyco-complexes[25,26]. The lines of carbohydrates inside the cells, involving D-mannose (Man), D-glucose (Glc), Glucuronic acid (GluA) and N-Acetyl-glucosamine (GlcNac) etc., occurred at 491 (Man), 551 (GlcNac), 564 (GluA, Glc), 597 (Man), 630 (GlcNac), 740 (GlcNac, Man, Glc, GluA), 768 (Glc), 922 (Man), 959 (Man), 980 (GlcNac), 1025 (GlcNac, Glc, GluA), 1042 (GluA, GlcNac), 1053 (Glc, GluA), 1073 (Glc, GluA, Man), 1102 (Man), 1120

(GluA, Glc), 1125 (GlcNac), 1143 (Man, GlcNac), 1204 (GluA, GlcNac, Glc), 1347 (Glc, GluA), 1371 (Glc), 1403 (Glc, GluA), 1457 (Glc, GluA, Man), 1557 (GlcNac), 1630 (GlcNac) and 1703 (GluA) cm^{-1}, etc.[27—29]. When the cells adhered, a number of the lines shifted and their intensities decreased or increased, for example, the line intensity of D-mannose at 491 cm^{-1} decreased clearly (the adhesive cells), whereas the line intensity of N-Acetyl-glucosamine at 550 cm^{-1} increased (two and three); the line intensity of glucuronic acid at 564 cm^{-1} increased obviously with the increase of number of adhesive cells and its line shifted to 571 cm^{-1} (two), 566 cm^{-1} (three) and 563 cm^{-1} (a group). The line intensity belonging to N-acetyl-glucosamine, D-mannose, glucose and glucuronic acid at 740 cm^{-1}decreased first (two and three) and then increased obviously (a group), whereas the line intensity of N-acetyl-glucosamine at 980 cm^{-1} increased first (two), then decreased (three and a group) clearly. The line intensity assigned to glucuronic acid, glucose and N-acetyl- glucosamine at 1204 cm^{-1} decreased considerably. It suggests that the groups of the carbohydrates have been modified.

2.4 The conformation of the lipids inside single and adhesive living cancer cells

Cellular adhesion takes place on cells membrane. The changes of conformation of cells membrane are interesting. Two lines at 2847 and 2877 cm^{-1} belonging to CH_2 symmetric and asymmetric stretching vibrations were sensitive to the change in structure of lipids bilayers of membranes[29,30]. The intensity ratio I_{CH_2} (I_{2877} / I_{2847}) reflects the state of leteral packing of liposomes. The order parameter for lateral interaction between chains can be calculated[29,30]. It was found that the order parameter decreased gradually with the increase of number of adhesive cells, which was 0.4 (single), 0.11 (two), 0.07 (three) and 0.01 (a group), respectively. So adhesion of cells caused an increase in fluidity of the membranes and ion permeability on the membranes[31,32].

To sum up, when cells adhered, the conformation of the biomolecules inside the cells had different changes: (i) the backbone of double-stranded DNA maintained orderly B-form or modified B-form conformation, whereas the groups of its deoxyribose and bases (A, G, C, T) were modified; (ii) the conformational changes of the main chain and the side chain in the protein were obviously variant. The lines intensity belonging to α-helix and β-sheet decreased, while that of β-turn increased. Tyrosine and tryptophane residues of the protein changed from "buried state" to "exposed state"; the lines intensity of its sulfhydryl group also increased; the conformation of its disulfide bond changed from two kinds to three kinds. These facts suggest that the cells adhesion causes changes in H-bonds organization of the main chain and environment of the side chain in the protein; (iii) the groups of the carbohydrates were also modified simultaneously; (iv) the conformation of the lipids bilayers of the membranes changed obviously; the order parameter for lateral interaction between chains decreased gradually with the increase of number of the adhesive cells. So cells adhesion resulted in an increase in fluidity of the membrane and ion permeability on the membrane. These results have started a prologue to expounding conformation changes of bio-

molecules in adhesive cells and laid down a foundation for studying the molecule mechanism of cell adhesion and cell-cell communication. Fox example: what are signal molecules which passed connective passageway of intercellular communication? In embryonic differentiation processes, how do the embryonic cells transfer the growth signals by cell-cell interaction? In the formation of tumour, because of the default of gap junction intercellular communication (GJIC), some cells possibly lose regulation of growth from normal cells, then what are the regulative factors of growth[10,11]? Therefor it is very important and beneficial to study the structure and relation between structure and function of these signal molecules and regulative factors for understanding the mechanism of cell-cell communication, morphogensis, and the formation of tumour, etc.

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