analytical chemistry

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NMR Spectroscopic Approach Reveals Metabolic Diversity of Human Blood Plasma Associated with Protein−Drug Interaction

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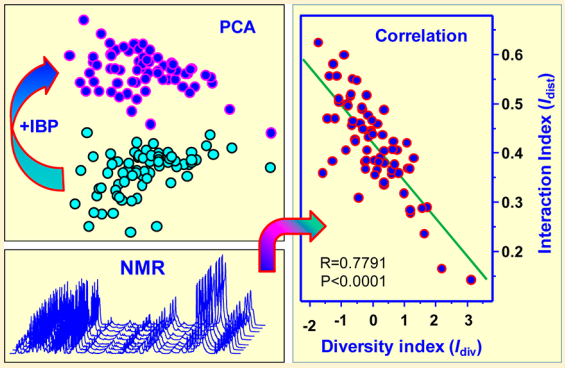
Supporting Information

ABSTRACT: Although blood plasma has been used to diagnose diseases and to evaluate physiological conditions, it is not easy to establish a global normal concentration range for the targeting components in the plasma due to the inherent metabolic diversity. We show here that NMR spectroscopy coupled with principal component analysis (PCA) may provide a useful method for quantitatively characterizing the metabolic diversity of human blood plasma. We analyzed 70 human blood plasma samples with and without addition of ibuprofen. By defining the PC score values as diversity index (I_{div}) and the drug-induced PC score value change as interaction index (I_{dist}), we find that the two indexes are highly correlated ($P < 0.0001$). Triglycerides, choline-containing phospholipids, lactate, and pyruvate are associated with both indexes ($P < 0.0001$), respectively. In addition, a significant amount of lactate and pyruvate are in the NMR "invisible" bound forms and can be replaced by ibuprofen. The diffusion and transverse relaxation time weighted NMR approaches gave rise to a better characterization of the diversity and the interaction than that of the one acquired using NOESYPR1D with 100 ms mixing time. These results might be useful for understanding the blood plasma−drug interaction and personalized therapy.



Blood plasma contains a large number of macromolecules, nutrients, and other metabolites, such as lipoproteins, albumin, antibodies, globulins, enzymes, lipid-based hormones, cytokines, amino acids, glucose, and organic acids and bases. Some of the components have been used as biomarkers or targets for diagnosing diseases and evaluating physiological conditions. Metabonomic studies of the plasma components have also been carried out for many years.[1−3] For example, the levels of low-density lipoprotein (LDL) and specifically oxidized LDL are correlated with the severity of atherosclerosis.[1,2] Blood plasma plays a key role in delivering drugs to body cells or organs. During the transportation process, complex interactions may happen between drug and blood proteins or metabolites.[3−9] These interactions may affect drug's pharmacological activity, distribution, and metabolism. However, the components and contents of blood plasma are usually varied with race, age, gender, genetic, nutritional status, living environment, and pathology condition.[3,9−12] Because of these variations, drug dosage or prescription may be different from one patient to another and from one season to another.[10] For most drugs, the fraction of drugs bound to plasma proteins, mainly albumin and α1-acid glycol-protein, varies greatly. For example, the plasma protein bound fraction of quinidine, a kind of antiarrhythmic activity drug, varies from 50% to 95% within different populations.[4,5] If the bound fraction of the drug is too low, patients might suffer from toxicity reaction, whereas if the bound fraction of the drug is too high, it might result in a reduction of therapeutic effect. In order to achieve maximum therapeutic effect, it is necessary to optimize dosage individually according to the interaction between the drug and the plasma proteins.[4,5] There is an increasing interest for personalized treatment according to the characteristics of an individual patient, in order to improve drug efficacy and reduce the number and severity of adverse drug reactions.[10] Because of the inter- and intraindividual variations of blood plasma, general normal concentration ranges of the biomarkers are commonly facing crisis, especially upper or lower limits of normal range. It had been reported that human metabolic phenotype diversity is associated with diet and blood pressure[13] and may require personalized drug treatment.[14,15] Therefore, it is essential to correlate the drug−protein interaction with diversity of blood plasma or other biological systems.

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for analysis of complex biofluids and drug−protein interaction in biological systems,[6,16−19] such as blood plasma/serum and urine. This is because NMR can provide

Received: April 16, 2013
Accepted: August 17, 2013
Published: August 19, 2013

 © 2013 American Chemical Society dx.doi.org/10.1021/ac401738z | *Anal. Chem.* 2013, 85, 8601−8608

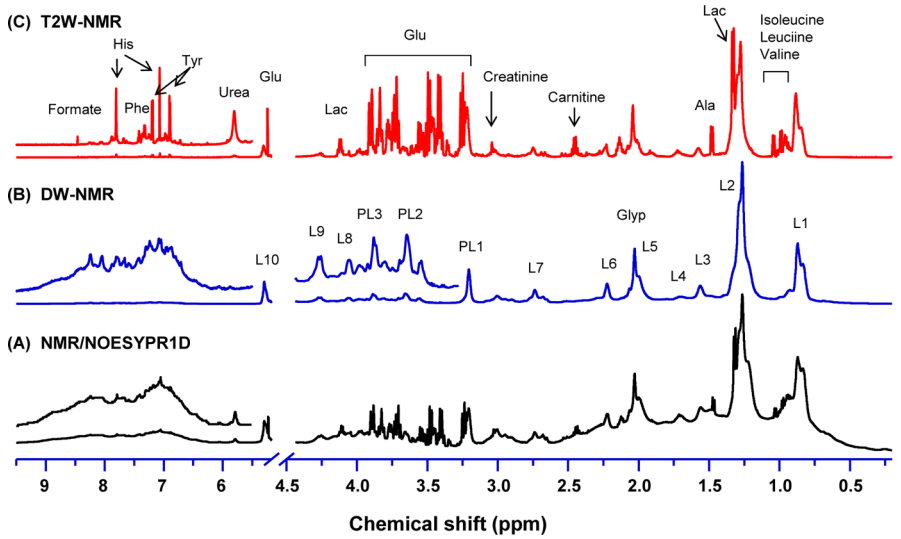


Figure 1. Typical 600 MHz ^{1}H NMR spectra of blood plasma acquired using NOESYPR1D (NMR) (A), DW-NMR (B), and T2W-NMR (C) approaches, respectively. The vertical scale of the region δ 5.17 to δ 10 was magnified 4-fold to emphasize low intensity signals. Assignment: L1−L10, resonances from lipoprotein fragments: L1, C$\underline{\mathbf{H}}_3$; L2, $(C\underline{\mathbf{H}}_2)_n$; L3, C$\underline{\mathbf{H}}_2CH_2$CO; L4, CH$_2C\underline{\mathbf{H}}_2$C=C; L5, C$\underline{\mathbf{H}}_2$C=C; L6, C$\underline{\mathbf{H}}_2$CO; L7, C=CC$\underline{\mathbf{H}}_2$C=C; L8 and L9, C$\underline{\mathbf{H}}_2$ of glycerol; L10, HC=C$\underline{\mathbf{H}}$CH$_2\underline{\mathbf{H}}$C=CH and =C$\underline{\mathbf{H}}$CH$_2$CH$_2$; PL1−3, -N$^+$(C$\underline{\mathbf{H}}_3$)$_3$, -C$\underline{\mathbf{H}}_2$N$^+$(CH$_3$)$_3$, and -O-C$\underline{\mathbf{H}}_2$-CH$_2$N$^+$(CH$_3$)$_3$ of choline-containing phospholipids in lipoprotein particles; Glyp, *N*-acetyls of glycoproteins; Lac, lactate; Ala, alanine; Glu, glucose; His, histidine; Tyr, tyrosine; Phe, phenylalanine.

compositional and structural information at molecular and atomic levels with high dynamic range in a noninvasion manner. In the past decades, many NMR methods have been established for studying drug−protein interaction, such as structure−activity relationships by NMR (SAR-by-NMR),[16] chemical shift interference,[20] saturation transfer difference[21,22] and NOE pumping,[17] diffusion rate interference,[18,19] and relaxation rate interference.[18,23] We have studied the low affinity and competitive binding of drugs to protein,[6,7,18,19,22,24−26] using diffusion and relaxation edited NMR approaches.[25−28] We studied the ibuprofen−protein interaction in intact blood plasma[6] and found that both ionic and hydrophobic interactions were involved.[6,7]

In addition to the NMR spectral editing, multivariate data analyses methods, such as principal components analysis (PCA), are good choices for analyzing complex NMR data. PCA is a well-known mathematical manipulation of a data matrix containing rows of samples and columns of variables,[29,30] which allows the expression of most of the variances within a data set in a small number of factors or principle components (PCs). The PCA result is visualized with a scores plot and the corresponding loadings plot, representing the differences of samples in different groups and the variables (metabolites) contributing to such difference, respectively. Each PC is a linear combination of independent terms such that each successive PC explains the maximum amount of variance possible, not accounted for by the previous PCs. Also, each PC is orthogonal and therefore independent of the other PCs.[29−31]

In this article we present an efficient NMR-PCA method to characterize the diversity of blood plasma and to associate the diversity with drug−plasma interaction. The method is demonstrated by using interaction of the intact blood plasma with ibuprofen (IBP), a non-steroidal anti-inflammatory drug with the potential function of suppressing oxidative modification of low density lipoprotein (LDL).[32] IBP also interacts with human serum albumin (HSA) and high density lipoprotein (HDL) in the intact human plasma.[6,7] We analyzed NMR spectra of 70 intact blood plasma samples from male volunteers with and without IBP and found that the PCA score values and the IBP-induced PCA score value changes can be used as diversity index and interaction index, respectively. The two indexes are highly correlated ($P < 0.0001$). The results reveal that metabolic diversity of blood plasma can be characterized by NMR in combination with PCA. The developed method here can also be used to predict drug−blood plasma interaction, which might be useful to guide personalized therapy.

EXPERIMENTAL SECTION

Materials. Human blood samples of 70 male volunteers were obtained by venipuncture into heparinized vacuum tubes. The plasma samples were separated conventionally by centrifugation (2000*g*, 15 min). Ibuprofen sodium salt was purchased from Sigma (Poole, Dorset, U.K.). Two 0.2-mL plasma samples were taken from each of the stored specimens and mixed with 0.3 mL of phosphate buffer prepared in D_2O (pD 7.4, 0.15 Mol/L). IBP was added in one of the samples to give a final concentration 5 mmol/L. This resulted in 70 pairs or two sets of samples with and without IBP, respectively. All samples were prepared at room temperature, and the whole procedure took about 2 h. The samples were then transferred into 5-mm tubes and stored in a refrigerator (−80 °C) before NMR measurement. In addition, concentrations of total triglycerides and lactate were assayed by using the enzymatic GPO-PAP[33,34] and the lactate oxidase[35] methods (Prodia Diagnostics), respectively, with an Olympus AU5400 apparatus. The relative contents of lactate, pyruvate, and the subunits of the lipids were measured from the NMR data (Supporting Information).

NMR Experiments. All NMR experiments were carried out on a Bruker Avance III 600 NMR spectrometer with the 5-mm inverse cryogenic probe (Bruker Biospin, Germany) operating at ^{1}H frequency of 600.13 MHz and at 25 °C. Three types of ^{1}H NMR spectra were collected for each of the samples

dx.doi.org/10.1021/ac401738z | *Anal. Chem.* 2013, 85, 8601−8608

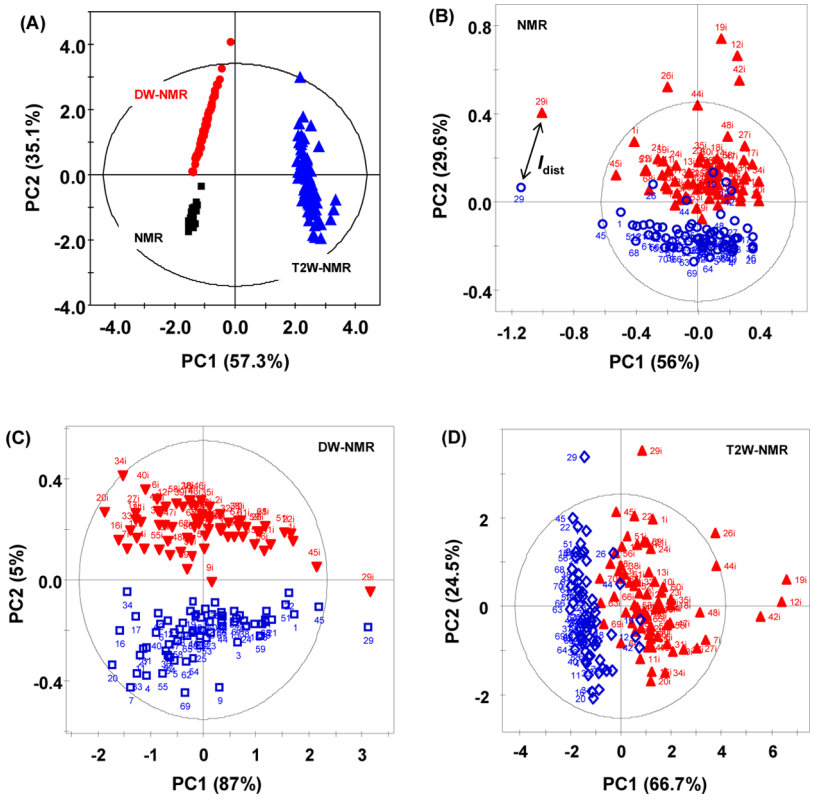


Figure 2. (A) PCA scores plots (PC1 vs PC2) based on the NMR (■), DW-NMR (red ●), and T2W-NMR (blue ▲) spectra of the blood plasma samples without IBP. Paired PCA based on the NMR (B), DW-NMR (C,) and T2W-NMR (D) data sets of the samples without (open symbols) and with (filled symbols) IBP. The IBP-induced PCA score change is labeled for one sample (in panel C) to show the definition of interaction index (I_{dist}).

covering a spectral range of 12019 Hz with 32 scans and 64 k data points: (1) NMR spectra were obtained using NOESYPR1D pulse sequence[36] with mixing time of 100 ms and repetition time of 4.88 s. (2) Diffusion-weighted (DW-NMR) spectra were obtained with water-suppressed bipolar gradient longitudinal eddy-current delay (BPPLED) sequence[37] with diffusion time (Δ) of 120 ms, eddy current recovery time (T_e) of 30 ms, gradient pulse duration (δ) and strength (G) of 3.2 ms and 19.4 G/cm, respectively. (3) Transverse relaxation time weighted (T2W-NMR) spectra were obtained using CPMG sequence[38] with total spin−echo time of 100 ms. Water resonance was suppressed using WATERGATE-W5.[39] It took less than 30 min from removal of the sample from the refrigerator to the end of NMR measurements.

Data Processing. The NMR spectra were processed conventionally using TOPSPIN 2.0 (Bruker Biospin). The time domain data were multiplied by an exponential function with line-broadening factor of 0.3 Hz and zero-filled to 128 k prior to Fourier transformation. The resonance of β-glucose-H^3 (δ 3.48) was used as an internal chemical-shift reference. The NMR spectra between δ 9.49 and δ 0.2 were integrated with equal width (1.8 Hz) using the AMIX package (V3.8.3, Bruker Biospin). The data within the region of δ 5.17−4.5 were removed to eliminate any possible baseline distortion caused by water suppression. Prior to PCA, each integration region was normalized to the total integration of the reduced digital spectrum. PCA was carried out using SIMCA-P 11.0 (Umetrics, Sweden) with mean-centered scaling type.

RESULTS AND DISCUSSION

Figure 1 shows typical 600 MHz ^{1}H NMR (A), DW-NMR (B), and T2W-NMR (C) spectra of one blood plasma sample without IBP. The resonances were assigned according to previous reports.[26,36,40] In the NMR spectrum (Figure 1A), the resonances from macromolecules (broad lines) and small molecules (sharp lines) are heavily overlapped. The spectra acquired using NOESYPR1D experienced a 100 ms T_1 weighting that affected the intensities of the resonances with T_1 less than 500 ms. The resonances from small molecules with large self-diffusion rates were efficiently suppressed in the DW-NMR spectrum (Figure 1B), and the remaining resonances were mainly from macromolecules with small diffusion rates, such as lipoproteins. In contrast, the T2W-NMR spectrum (Figure 1C) contained resonances with slow relaxation rate, which were mainly from small molecular components, such as amino acids and organic acids, and the flexible groups of lipoproteins, while the broad peaks of macromolecules were attenuated.

The NMR, DW-NMR, and T2W-NMR data sets of the intact plasma samples without IBP were statistically analyzed using PCA. The scores plot (Figure 2A) shows that the DW-NMR and T2W-NMR experiments give rise to larger dispersions compared to that from the NMR. The corresponding loadings plot (Supplementary Figure S1) shows that the separation of the three experimental data sets is due mainly to the content differences of the macro- and small molecules, as expected. To find out if the PCA values can be used to characterize the diversity of the samples and IBP-induced metabolic profile change, we performed a paired PCA for each

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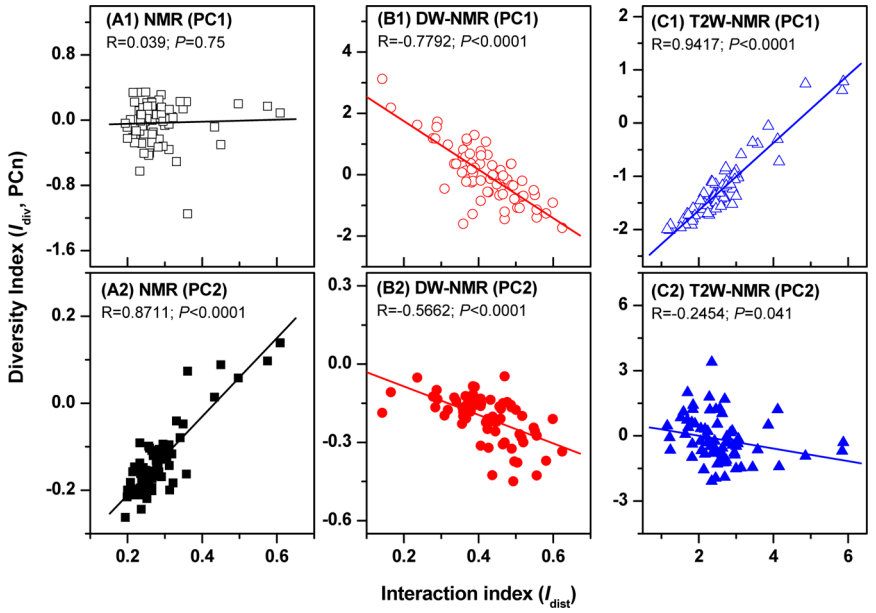

Figure 3. Correlations between the diversity indexes (PC1, PC2) and the interaction indexes (location change from PC1/PC2 to $PC1_i/PC2_i$) derived from the PCA of (A1, A2) NMR, (B1, B2) DW-NMR and (C1, C2) T2W-NMR data sets of the samples with and without IBP. The linear fitting parameters (R, P) are given.

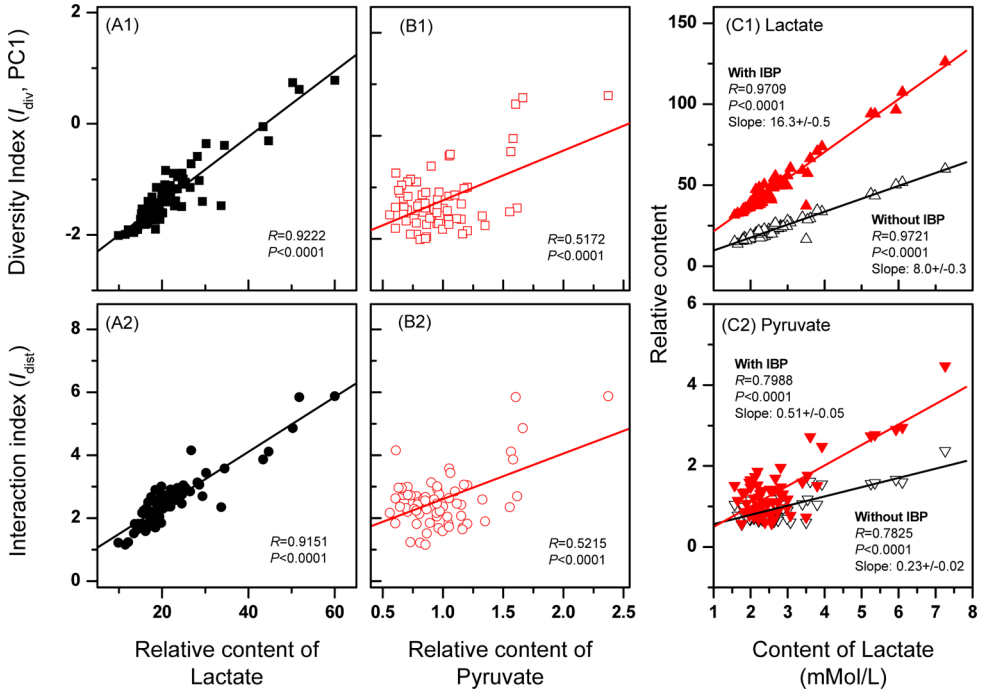

Figure 4. Plots of the diversity indexes (A1, B1) and the interaction index (A2, B2) as function of the relative contents of lactate (A1, A2) and pyruvate (B1, B2), respectively. (C) Correlations between the contents of lactate and the relative contents of lactate (C1) and pyruvate (C2) without (open symbols) and with IBP (filled symbols). Linear fittings (line) and the fitting parameters (R, P) are also given.

type of the experiments on the samples with and without IBP, respectively. The PCA scores plots and the linear loadings plots are shown, respectively, in Figure 2B–D and in Supplementary Figure S2. It had been shown that the NMR peaks of IBP at 5 mmol/L were too broad to be observed as the drug is fully bound to the macromolecules in blood plasma.[6,7] This is further confirmed by careful visualizing the spectra in the current study. In addition, we did not observe any peak of IBP in the loadings plots (Supplementary Figure S2). Therefore, the separations shown in Figure 2B–2D are dominated by the drug-induced metabolic profile changes. Since the first two PCs represent more than 85% of the observable variants (Figure 2), PC1 or PC2 is defined as diversity index (I_{div}) to represent the metabolic profile. The IBP-induced PCA score values change or the distance moved is defined as the interaction index (I_{dist}) to measure the strength of the drug–plasma interaction:

$$I_{dist} = \sqrt{(PC1 - PC1_i)^2 + (PC2 - PC2_i)^2} \quad (1)$$

dx.doi.org/10.1021/ac401738z | Anal. Chem. 2013, 85, 8601–8608

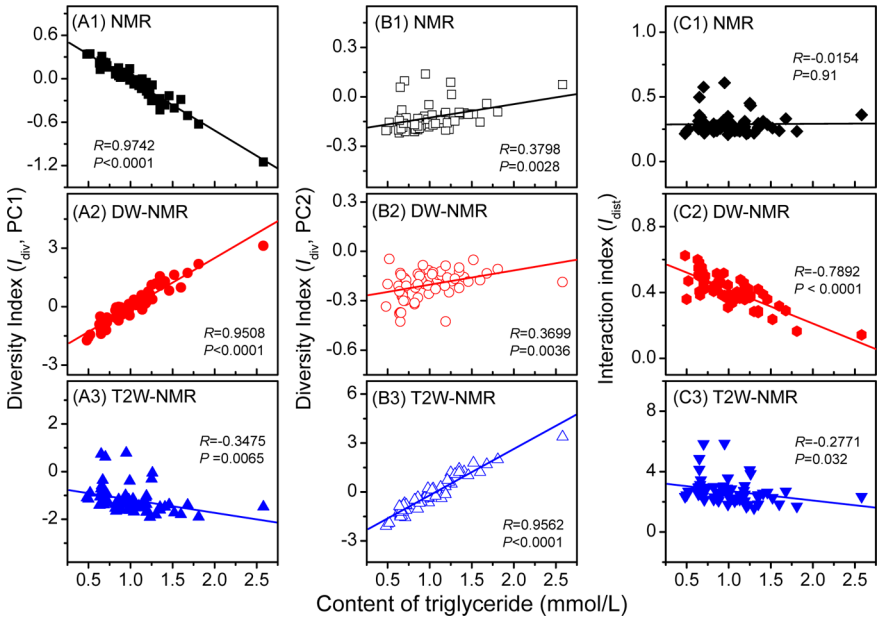

Figure 5. Plots of the diversity indexes defined by PC1 (A1-A3) and PC2 (B1−B3), and the interaction index (C1−C3) as function of the content of triglycerides, based on PCA of NMR (A1, B1, C1), DW-NMR (A2, B2,C2), and T2W-NMR (A3, B3, C3) data sets. Linear fittings (line symbols) and the fitting parameters (R, P) were also given.

where PC1/PC2 and $PC1_i/PC2_i$ represent the PCA score values for each of the samples before and after addition of IBP, respectively.

Figure 3 shows the correlations between I_{div} and I_{dist} derived from the PCA score values of Figure 2B−D. The significant linear correlations are obtained between the two indexes (P < 0.05, Figure 3) with exception of I_{div} (PC1) of the NMR experiments (Figure 3A1). These results indicate that the NMR-PCA approach can be used to characterize the metabolic diversity or profile of the blood plasma and the IBP−plasma interaction. Referring to the PCA loadings plots (Supplementary Figure S2), we know that the IBP-induced changes include the relative intensity increments of lactate, pyruvate, and phenylalanine and the upfield chemical shift drift of choline-containing phospholipids as the dispersive line shapes (Supplementary Figure S2), which is consistent with our previous results.[6,7] Interestingly, the contributions of the lipid fragments (L1−L10) and the choline-containing fragments (PL1, PL2, $(CH_2)_2$, and CH_3 at high field regions) are opposite. In addition, IBP-induced chemical shift changes may also contribute to the values of both I_{div} and I_{dist}.

To quantitatively investigate the relationships between the contents of the plasma metabolites and I_{div} or I_{dist}, we measured the levels of triglycerides and lactate of 60 samples and the relative contents of lactate, pyruvate, and structural units of lipid chains $(CH_2)_n$, fatty acyl group CH_2CO, and choline headgroup $-N(CH_3)_3$ of 70 samples from the T2W-NMR and DW-NMR spectra, respectively. The methods used for the content measurement are presented in the Supporting Information.

Addition of IBP into the blood plasma causes intensity changes of a number of small molecules, such as lactate, pyruvate, glucose, tyrosine, histidine, and phenylalanine (Supplementary Figure S2). Among those molecules, lactate experiences the largest variation in content. Figure 4A and B show that the relative contents of lactate and pyruvate are highly correlated (P < 0.001) with both indexes, I_{div} and I_{dist}, derived from T2W-NMR data set, respectively. It is known that lactate is a most abundant small molecule in the blood and involves several metabolic pathways and physiological processes; for example, during physical exercises, glucose is broken down and oxidized to pyruvate and then to lactate.[41] It is also known that about two-third of the blood lactate is in the NMR-"invisible" bound form.[6,42−44] Upon addition of the high affinity IBP into the blood plasma, the bound lactate is replaced, resulting in dramatic increment (114 ± 12%) of the peak intensities (Figure 4C1). Since the free and bound forms of lactate have different relaxation times and are in exchange, the relaxation time variation is expected to be larger than the ones presented in unit form. We found that the lactate contents measured are linearly correlated with the relative contents derived from T2W-NMR spectra (Figure 4C1, R > 0.97, P < 0.0001, N = 60). This means, at least for lactate, the effect of the relaxation time variation on the relative content derivation is negligible under the current experimental setup. Pyruvate is an important chemical compound in biochemistry. It is the output of the anaerobic metabolism of glucose known as glycolysis,[41] although the concentration of pyruvate is about 30-fold lower than that of lactate. We still observed good correlation between the relative content of pyruvate and of both the diversity index and the interaction index (Figure 4B). Similar to lactate, the relative contents of pyruvate in the presence of IBP are significantly increased (39 ± 23%, Figure 4C2), which means that at least ~40% of pyruvate is in the NMR-"invisible" bound form and released upon addition of IBP. Tyrosine, histidine, and phenylalanine may also contribute to the dispersion of the data as shown in Supplementary Figure S2. However, further analysis indicates that there are no correlations between the contents of those metabolites and the diversity and interaction indexes derived from PC1 and PC2. From Figure 4A and B, we may conclude that the content variations of the plasma lactate and pyruvate contribute to the diversity and the competitive binding between IBP and lactate

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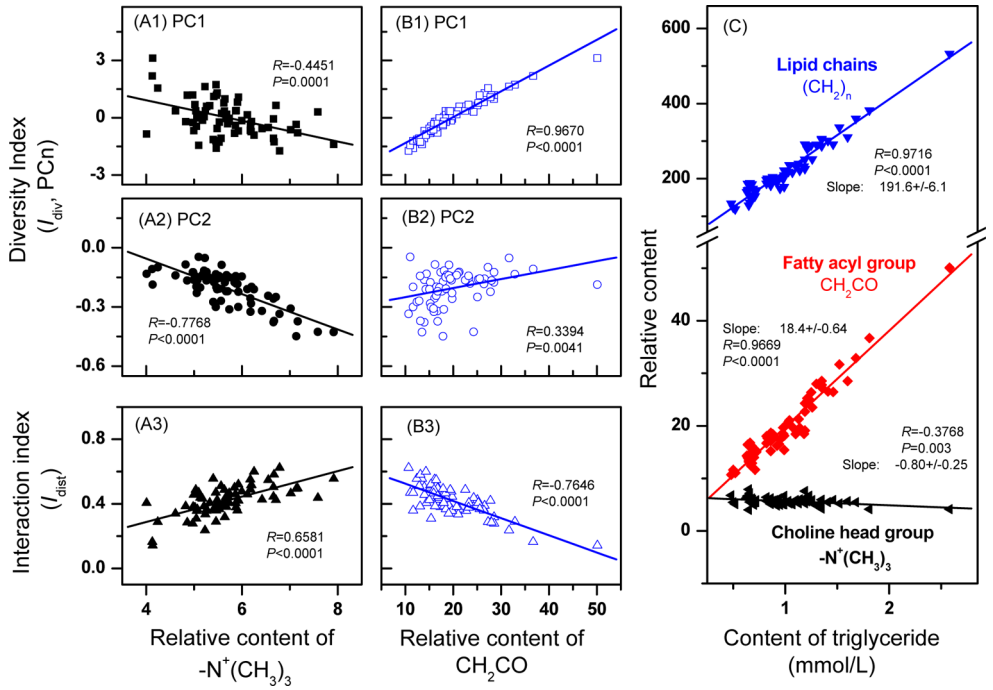

Figure 6. Plots of the diversity indexes (A1, A2; B1, B2) and the interaction index (A3, B3) as function of the relative contents of the choline headgroup $-N^+(CH_3)_3$ (A1−A3) and fatty acyl group CH_2CO (B1−B3). (C) Relationships between the contents of triglyceride and the relative contents of lipid chains $(CH_2)_n$ (blue ▼), fatty acyl group CH_2CO (red ◆), and choline headgroup $N^+(CH_3)_3$ (◀), respectively. Linear fittings (line) and the fitting parameters (R, P, slope) are also given.

or pyruvate on the plasma proteins, mainly albumin,[6,42−44] is associated with the IBP−plasma interaction.

Lipoproteins are the main macro-components in blood plasma and are divided into several subclasses, such as HDL, IDL, LDL, and VLDL, according to their size and density. The lipoproteins are generally spherical in shape with the layers consisting of different phospholipids and the core containing triglycerides, cholesterol, and cholesterol esters.[26,45] The lipid chains $(CH_2)_n$ and fatty acyl group (CH_2CO) are typical structural units of the phospholipids, triglycerides, and cholesterol ester. Figure 5 shows the relationships between the contents of triglycerides and the two indexes, I_{div} and I_{dist}, derived from NMR, DW-NMR, and T2W-NMR, respectively. Excellent correlations (Figure 5, A1, A2, B3. $R > 0.95$, $P < 0.0001$, $N = 60$) are obtained between the contents of triglycerides and the diversity indexes that associate mainly with the variants of the macromolecules (Supplementary Figure S2A1, B1, C2). A similar result is obtained between the two indexes and the relative contents of the lipid chains $(CH_2)_n$ (Supplementary Figure S3). As expected, the PCA based on the DW-NMR data set gives rise to a better characterization of the macro-molecule-related diversity and interaction. Therefore, we show only the correlation between the relative contents of the choline headgroup $-N^+(CH_3)_3$, the fatty acyl group CH_2CO, and the two indexes derived from DW-NMR data sets in Figure 6A and B, respectively. Because the splitting along PC2 (Figure 2C) is mainly associated with the IBP-induced chemical shift changes (Supplementary Figure S2B2) of the choline-containing phospholipids, the diversity index defined by PC2 shows a better correlation with the relative content of the choline headgroup (Figure 6A2). We had observed the hydrophobic and hydrophilic interaction between IBP and the two abundant phospholipids, phosphatidylcholine and sphingomyelin.[6,7] Furthermore, the unsaturated protons (L10, -HC═C; L5, $-CH_2C$═C), as well as $-OCH_2$ of choline, are also experienced upfield chemical shift drift (Supplementary Figure S2B2). The results reveal that the whole structures of the choline-containing phospholipids may be involved in the interaction. Considering the intermolecular cross-peaks observed in NOESY spectra,[7] we could conclude that IBP may insert into the lipoprotein layer, which may be an important factor for IBP as a potential anti-oxidation agent of lipoproteins.[32]

It is noticed that the contents of triglycerides (Figure 5C2) and the relative contents of $(CH_2)_n$ (Supplementary Figure S3C) and CH_2CO (Figure 6B3) show negative correlation with the interaction index, but that of $-N^+(CH_3)_3$ shows positive correlation (Figure 6A3). These observations are consistent with the results (Figure 6C) that the contents of triglycerides are positively correlated with the relative contents of $(CH_2)_n$ and CH_2CO ($R > 0.96$, $P < 0.0001$) but negatively correlated with that of $N^+(CH_3)_3$ ($R = -0.38$, $P = 0.003$). There are two possible explanations for the phenomena. First, IBP interacts with choline-containing phospholipids, such as phosphatidylcholine and sphingomyelin,[6,7] resulting in the chemical shift upfield drift for the choline headgroup $-N^+(CH_3)_3$ and the other fatty acyl group protons, as indicated by dispersive line shapes in Supplementary Figure S2. The phospholipids are located in the layer of lipoproteins. The small lipoproteins (such as HDL) have higher binding affinity to IBP and experience larger chemical shift changes in the presence of IBP compared to the large ones (such as LDL).[7] These lead to a positive correlation between the contents of $N^+(CH_3)_3$ and the interaction index. Second, triglycerides, as well as cholesteryl esters, are mainly located within the core of the lipoproteins and therefore do not interact with the IBP. The large lipoproteins contain more triglycerides and cholesteryl esters in the core and less choline-containing phospholipids in the

dx.doi.org/10.1021/ac401738z | Anal. Chem. 2013, 85, 8601−8608

layer than small lipoproteins.[7,45] High contents of nonbinding triglycerides, possible cholesteryl esters as well, intend to minimize the IBP-induced plasma profile change. This may explain the negative relationship between the content of triglycerides and the interaction index. As CH_2CO and $(CH_2)_n$ are the main structural units of the triglycerides and cholesteryl esters, their contents are also negatively associated with the interaction index. However, contents of triglycerides, CH_2CO, and $(CH_2)_n$ contribute to the diversity indexes. Although the contents of triglycerides are highly correlated with that of CH_2CO and $(CH_2)_n$ (Figure 6C), we could not conclude that CH_2CO and $(CH_2)_n$ belong purely to triglyceride since there are many other long chain lipids in the blood plasma. These results demonstrate that the DW-NMR experiment may be a choice for characterizing macromolecule-related diversity and interaction of blood plasma.

It is known that IBP interacts with a number of plasma proteins,[6,42,44] such human serum albumin, α1-antitrypsin, transferrin, lactate dehydrogenase, etc. In a previous study on the interaction of IBP with HDL and LDL, we did not observe the NMR signal of lactate in the presence or absence of IBP.[7] HDL and LDL may be excluded from the lactate-binding plasma protein matrix. In addition, the interaction may also be affected by the small molecular weight metabolites in the plasma as the result of competitive binding. Detailed studies on the contribution of the specific plasma proteins to the diversity, IBP–plasma interaction, and the influence of the other metabolites on both the diversity and the interaction are needed. It is worth to note that the diversity is general, but the drug–plasma interaction is specific. Which blood plasma component is involved in the interaction depends on the particular drug used.

CONCLUSIONS

It is demonstrated that NMR spectroscopy, especially DW-NMR and T2W-NMR, combined with PCA could be used for characterizing blood plasma diversity and the drug–plasma interaction. Seventy blood plasma samples and IBP are used as a model system. We define PCA score value (PC1 or PC2) without IBP as the diversity index and the PCA score change induced by IBP as the interaction index for the each of the samples. We find that the relative contents of triglycerides, choline-containing phospholipids, lactate, and pyruvate are highly correlated with the diversity and the interaction indexes. Although only 70 blood plasma samples were studied and the drug was added directly into the samples, the results presented here show that NMR is a potential approach for characterizing diversity and molecular interaction in a complex biosystem.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is supported by the grants from 973 Project of China (2009CB918603) and National Natural Science Foundation of China (nos. 21275157, 21120102038, 21075132, 21221064).

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