Towards an Atlas of the zebrafish metabolome by ^{1}H-NMR

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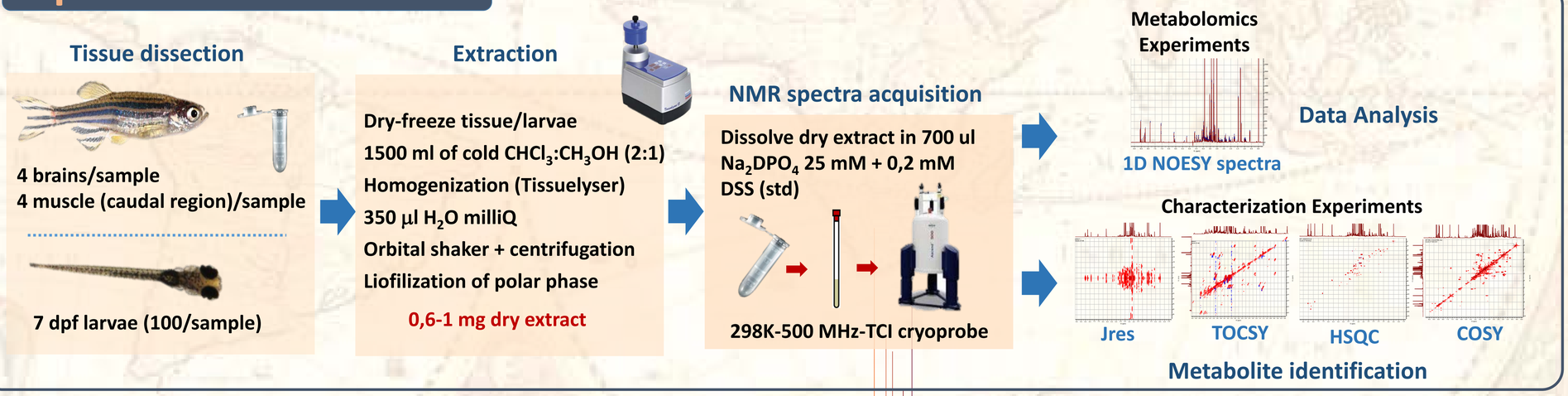
Introduction

Metabolomics studies the metabolic composition in cells, tissues, organs or whole organisms. Since it is closer to phenotypes than transcriptomics and genomics[1], it constitutes a bridge between purely molecular events and macroscopic phenotypes. Metabolome analysis using one-dimensional proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy has many strengths (low sample preparation, non-destructive, inherently quantitative); however, its application has been hindered by its lower sensitivity and dynamic range, when compared with Mass Spectrometry (MS). Current advances in NMR instrumentation (higher magnetic field instruments, use of cryoprobes) constituted a substantial sensitivity improvement, thus leading to similar results for both techniques[2]. Zebrafish models are extensively used in vertebrate biology, drug development and (eco)toxicology[3]. While zebrafish genetic and gene-expression analysis benefit from the existence of several molecular tools, and the transparency of its embryos promoted the development of highly sophisticated imaging techniques, zebrafish biochemistry and, specifically, the study of its metabolomic profile is still lagging behind. Our recent work found that even a partial description of the metabolome can be of enormous help in describing toxic effects related to molecular events, like endocrine disruption[4]. Here we present introductory analysis of the zebrafish metabolome using NMR spectroscopy.

Objectives

- To adjust preparative and analytical methods to extract metabolites from zebrafish larvae, adult brain and muscle.
- To characterize the metabolomes of ZF larvae, adult brain and muscle by NMR spectroscopy.

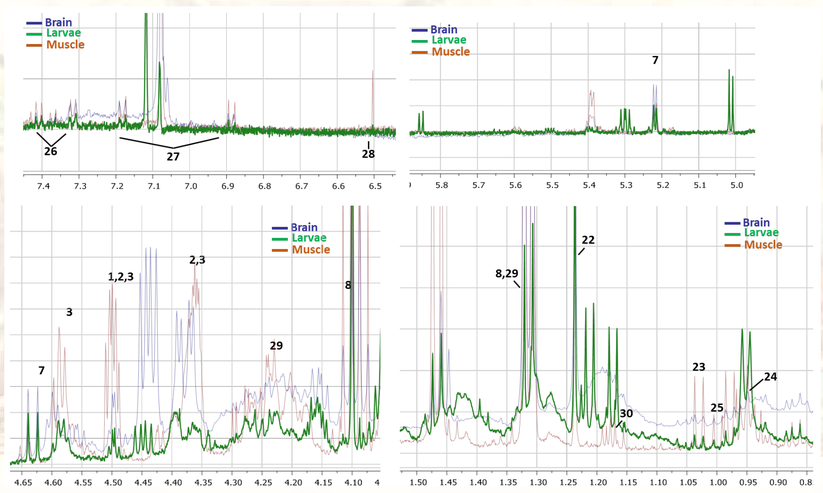
Experimental Workflow



Results

Metabolite identification

1a



1b

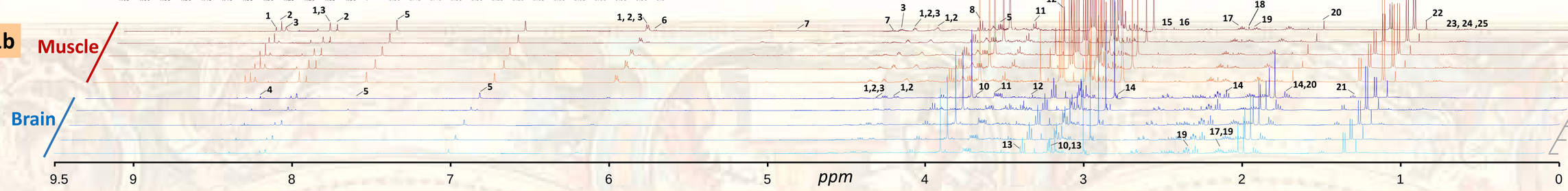


Figure 1a: Superimposed ^{1}H NMR 500 MHz spectra of larvae (green), muscle (maroon) and brain (blue)

Figure 1b: Stack plot of ^{1}H NMR spectra of 5 replicates of muscle extract (maroon) and brain extract (blue).

Metabolite assignment: 1. AMP ; 2. Inosinic Acid; 3. ATP; 4. Formate; 5. L-Histidine; 6. NAD; 7. D-Glucose; 8. Lactate.; 9. Creatine; 10. Betaine; 11. H-AA (L-Alanine, L-Glutamine, L-Glutamic Ac, etc); 12. L-Glycine; 13. Taurine; 14. GABA; 15. L-Asparagine; 16. L-Aspartate.; 17. L-Glutamine; 18. Succinic Ac.; 19. L-Glutamate; 20. Acetic Ac.; 21. L-Alanine; 22. Fatty Ac.; 23. L-Valine; 24. L-Leucine; 25. L-Isoleucine; 26. L-Phenylalanine; 27. L-Tyrosine; 28. Fumarate; 29. L-Threonine; 30. Ethanol

Data Analysis

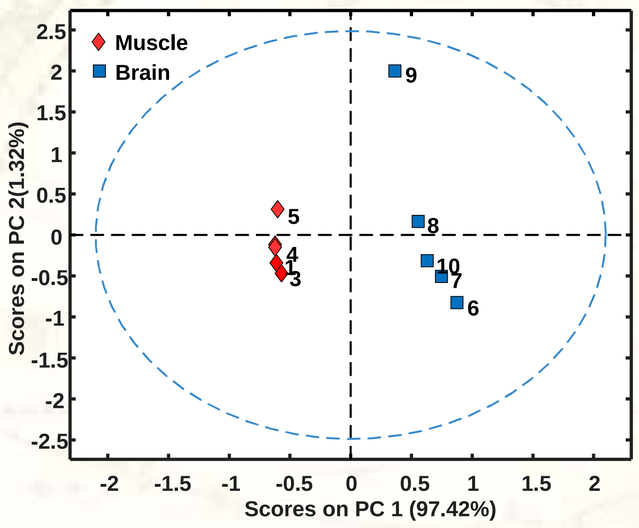


Figure 2: PCA score plots of the ^{1}H NMR spectra from metabolic extracts of *ZF* muscle and brain.

Table 1: List of preliminary NMR assignment in muscle (M), brain (B) and larvae (L).

Metabolite	Spectral asigment	M	B	L
Acetate	1.9 (s)	+	+	+
L-Alanine	3.76(q), 1.46(d)	+	+	+
L-Arginine	3.76(t), 3.23(t), 1.90(m)	2	n.d.	2
L-Asparagine	4 (dd), 2.96(dd), 2.87(dd)	+	1,2	+
L-Aspartate	3, 89 (dd), 2.80(dd), 2.68 (dd), 2.65 (dd)	+	+	+
AMP	8.58 (s), 8.26(s), 6.12(d), 4.5(dd), 4.36(dd), 4.01(dd)	+	+	+
ATP	8.52(s), 8.26(s), 6.13(d), 4.6(t), 4.5(m), 4.39(m), 4.28(m)	+	+	+
Betaine	3.89(s), 3.25(s)	1	1	1
Citrate	2.67(d), 2.64(d)	2	2	n.d
Choline	4.06(ddd), 3.51(dd), 3.19(s)	+	+	+
Creatine/P-Creatine	3.92(s), 3.02(s)	+	+	+
D-Glucose	5.22(d), 4.63(d), 3.89(dd), 3.82(m), 3.73(m), 3.52(dd), 3.395(m), 3.23(dd)	+	+	+
D-Glucose-6-phosphate	5.22(d), 4.63(d), 4.04(m), 3.95(ddd), 3.48(d), 3.28(dd)	+	+	+
Ethanol	3.63(q), 1.17(t)	+	2	+
Fatty Ac.	1.24(s)	+	+	+
Formate	8.44(s)	+	+	+
Fumarate	6.5(s)	1	1	1
Gamma-Aminobutyric acid	3,0(t), 2.28(t), 1.89(m)	n.d.	+	+
L-Glutamate	3.75(dd), 2.34(m), 2.12(m), 2.05(m)	+	+	+
L-Glutamine	3.76(t), 2.45(m), 2.12(m),	+	+	+
L-Glycine(s)	3.54(s)	+	+	+
L-Histidine	7.86(s), 7.08(s), 3.98(dd), 3.23(dd), 3.16(dd)	+	+	+
Hypoxanthine	8.2(s),8.18(s)	n.d.	n.d.	1
Inosinic Ac	8.55 (s), 8.21(s), 6.12(d), 4.5, 4.36, 4.01	+	+	+
L-Isoleucine	3.65 (d), 1.45(m), 1.01(d), 0.93(t)	+	n.d.	+
Lactate	4.10 (q), 1.32 (d)	+	+	+
L-Leucine	3.72(m), 1.7(m), 0.95(t)	+	2	2
L-Lysine	3.74(t), 1.89(t), 1.71(m), 1.45(m)	+	+	n.d
Malate	4.29(dd), 2.66(dd), 2, 36(dd)	+	2	2
NAD^+	9.33(s), 9.15(d), 8.83(d), 8.42(s), 8.20(m), 6.08(d), 6.02(d)	+	+	n.d
Niacinamide	8.92(s), 8.70(dd), 8.24(dd), 7.58(dd)	n.d.	n.d.	+
L-Phenylalanine	7.42(m), 7.32(d)	+	+	+
Succinic Ac	2.39(s)	+	+	+
Taurine	3.41(t), 3.25(t)	+	+	+
L-Threonine	4.24(m), 3.56 (d), 1.31 (d)	2	2	2
L-Tyrosine	7.17(m), 6.89(m)	+	+	+
L-Valine	1.03 (d), 0.97(d)	+	+	+

n.d.: Non detected in the extract; 1: Assignment only in ^{1}H NMR; 2: Overlapping with other metabolites.

Conclusions

- Resonances from a total of 36 metabolites have been assigned in the ^{1}HNMR spectral dataset. Detected metabolites are in agreement with those described in literature[5-8]. In addition, for most of them, metabolite identification was confirmed from COSY, TOCSY, HSQC and J-res spectra of representative samples.
- The amount of tissue required is not dramatically larger (1-5 fold) than for MS metabolomics or transcriptomics analyses.
- Preliminary evaluation of ^{1}H NMR fingerprints of muscle and brain extract allows a perfect separation of both tissues. Muscle extracts contain more Lactate, L-Alanine, Creatine, Taurine and ATP/AMP while brain extracts show more GABA and L-Aspartate, among others.

Future prospects

- A more profound characterization of the ZF metabolome will be carried out by A) identifying the unassigned proton resonances in more concentrated samples and with spiking experiments and B) by further quantitation of the obtained data.
- We plan to use this technique to evaluate the effects of xenobiotic exposure on the ZF metabolome. This knowledge will contribute to clarify the mechanisms that connect initial molecular events (e.g. interaction of a xenobiotic with a molecular receptor) and the observed organism phenotype

References

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