Dymension 2D Gel Analysis Software Helps Detect Proteins Affected by Anti-addictive Compound Ibogaine

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Introduction

Ibogaine, an indole alkaloid present in the root of plant Tabernanthe iboga has been shown to have anti-addiction properties against opiates[1], [2], stimulants[3] and alcohol[4]. Since the anti-addiction effect lasts longer than the presence of ibogaine in the body, metabolic changes are occurring in the brain which could be studied using a 2D gel based proteomics approach. The protein profiles could then be used to establish which proteins are up or down-regulated and may lead to a better understanding of the pharmacodynamics of anti-addiction therapies. However, to be able to detect which proteins in a complex 2D gel image are being expressed requires sophisticated software to resolve protein spot images quickly and easily. To overcome the analysis hurdle, this article describes how Dymension, a 2D gel image analysis software with innovative algorithms can be used and the results the software can achieve when analysing proteins from rat brains after ibogaine treatment.

Materials and methods

Proteins (150 µg per sample) extracted from the brains of 6 rats treated with ibogaine (20 mg/kg) at 24 and 72 hours or 6 rats treated with water as a control also at 24 or 72 hours were mixed with rehydration solution (9 M urea, 2% (w/v) CHAPS, 2% (v/v) immobilized pH gradient (IPG) buffer, 18 mM DTT, a trace of bromophenol blue). The proteins were loaded onto immobilized pH 3–10 non-linear gradient strips followed by 12% SDS-PAGE gels and run under standard 2D gel electrophoresis conditions on a Multiphor II (GE Healthcare, Little Chalfont, UK) and a Hoeffer SE 600 (GE Healthcare, Little Chalfont, UK). The resulting gels were silver stained using a protocol5 compatible with matrix-assisted laser desorption/ ionization–time of flight mass spectrometry (MALDI-TOF MS). Images of the 2D gels were produced using an Artixscan 1800f scanner (Microtek, Carson, CA, USA). Gel image analysis was performed with Dymension 2 software (Syngene, Cambridge, UK). Dymension 2 is suitable for determining the amount of protein present, before and after ibogaine induction treatments because the software automatically detects and assigns statistical confidence to each and every difference in spot normalised volume, to accurately highlight proteins of interest. In this study, Dymension compared the treated top the control sample based on the average of the replicates in each sample. For all spot intensity calculations, normalised volume values were used and the results were expressed as the ratio of the normalised volume of a protein spot in ibogaine-treated rats divided by the normalised volume of matched protein spot in control rats. The results of the analysis were displayed as a table, 3D spot profile, bar chart, correlation and scatter plot which made it easy to compare many expression profiles simultaneously and to pick proteins for further analysis.
The protein spots that showed significant changes in intensity compared to the controls were excised from the separate gels and analysed by MALDI–TOF MS using Voyager DE-STR instrument at the Aberdeen Proteome Facility (University of Aberdeen, Aberdeen, Scotland). The Mascot software was used to search NCBInr database to identify the proteins.

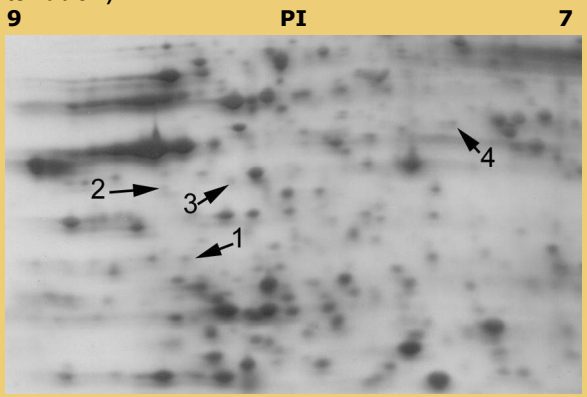
Results

The Dymension analysis of 2D gels containing proteins from ibogaine-treated rats compared to control rats detected 12 protein spots which showed significant changes in intensity. After further MALDI–TOF MS analysis, four proteins were identified as glyceraldehyde-3-phosphate dehydrogenase, aldolase A, pyruvate kinase and malate dehydrogenase, all metabolic enzymes involved in glycolysis and the tricarboxylic acid (TCA) cycle. 24 hours after ibogaine treatment the levels of these enzymes were only slightly above the control values, between 1.1- and 1.4-fold increases (Table 1). Changes in protein expression were most significant at 72 hours after ibogaine administration when spot intensities of the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, aldolase A, and pyruvate kinase were increased about 3.2-, 2.5-, and 2.9-fold, respectively. The amount of one enzyme from the TCA cycle, malate dehydrogenase, was increased around 3.6-fold (Table 1, Figures 1 and 2). This could explain ibogaine's prolonged action in the body but further work is needed to determine the explanations of ibogaine action on humans.

Table 1: Proteins induced 24 and 72 hours after ibogaine treatment

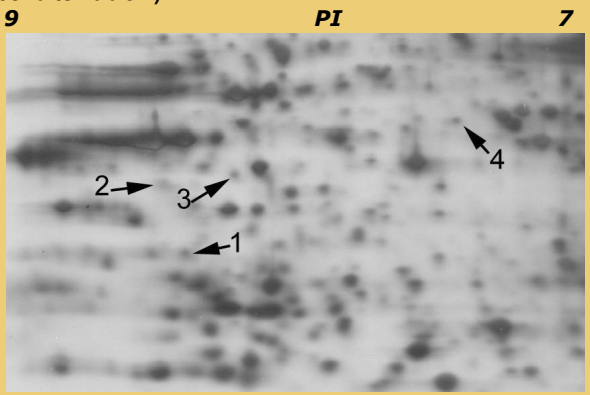
Spot /enzyme	Accession number	Fold over control (24hr)	Fold over control (72hr)	Theoretical Mr(Da)/pI	Score	Matched Peptides	Sequence coverage (%)
1 Glyceraldehyde -3-phosphate	Q9QWU4	1.13	3.21	36,090/8.14	62	9	36
2 Malate Dehydrogenase	42476181	1.42	3.64	17/8.79	54	9	30
3 Aldolase A	6978487	1.23	2.45	39,783/8.05	60	9	24
4 Pyruvate kinase	206205	1.38	2.94	58,314/7.19	70	10	26

Figure 1: Partial 2-D gel images of rat brain proteome 72 hour post treatment with control (numbers correspond to Table 1).



Results

Figure 2: Partial 2-D gel images of rat brain proteome representative of a single treatment with ibogaine 72 hours after treatment (numbers correspond to Table 1).



Conclusions

Using Dymension 2D gel analysis software in conjunction with MALDI-TOF MS has enabled the detection of changes in four metabolic enzymes at both 24 and 72 hours post ibogaine treatment. The software's ability to perform complicated analysis of four sets of triplicate gels with ease, demonstrates that Dymension 2 is an excellent, rapid method of determining the proteomic mechanisms of drug action.

References

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