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Raj’s email: Thu 12/1/2016 10:54 AM

I've reviewed the report so far; please address the points below in addn to the ones you are currently addressing, and also add the following comments to wiki for future reference:

--quoting the product formation in 30 mins in terms of % substrate converted is arbitrary since it depends on the substrate concentration. Hence please provide the product formation in terms of moles and also provide next to it the number of moles of enzyme used in the respective experiments.

AU: I reported uM product produced in the table. Now pmole formed will be included. Sirt3 concentrations in terms of uM will be included in the table.

--given the results with NAM inhibition of MnSOD and FdL2, we see that stopping the reaction with NAM for the purpose of MST experiments on substrate binding is more effective for FdL2. Thomas is currently doing some MST experiments with unlabeled FdL2.

If we want to use NAM to stop reaction in MST experiments (as opposed to or in addition to carbaNAD approach to getting Kd of NAD), we may want to use unlabeled FdL2 instead of MnSOD.

AU: Question: does these two method of stopping the reaction has different mode of action? Or does it matter what mode of inhibition used to stop the reaction and how does it affect MST data?

In this regard, I may ask you to check rate of reaction for unlabeled FdL2 peptide in presence of fully inhibitory [NAM] using HPLC in near future.

AU: XG please comment.

We will do the MST expt after that.

(Note: Alternatively, one could measure the rate of NAD conversion during such MST experiments, it would be more accurate to monitor reaction with Sirtainty, since it measures the rate of NAD conversion.

However, problem is that this cannot be done in presence of NAM inhibitor.)

--BTW, in the future, one of you should do initial rate fitting with/without 120 min or last two time pts (misc task) to see whether they affect. Not relevant for current expts, but could save enzyme in future.

AU: OK, thanks

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Email from Raj: Wednesday, November 30, 2016 1:42 PM

Have reviewed the data. Please answer all following on wiki as appropriate.

1) Seems IC50,NAM is about 4-5x higher than that for FdL2 peptide (IC50 there I assume was also measured at 3000uM NAD).

AU: As per XG, the IC50 NAM (36.8 uM) on FdL peptide was determined with 3000 uM NAD, 100 uM FdL peptide with 5U/rxn Enzo Sirt3, using Fluoroskan.

Note we seem to observe partial inhibition of around 70% at saturating NAM (though this is an estimate). We don't want to get into the saturating range in our initial rate experiments, of course, but that shouldn't be a problem. Does Guan have the partial inhibition at saturating NAM for FdL2 peptide handy? If so it can posted side-by-side.

AU: XG’s data- with 3000 uM NAD, 100 uM FdL peptide with 5U/rxn Enzo Sirt3, using Fluoroskan.

|  |  |
| --- | --- |
| **[NAM],uM** | **% Activity** |
| 200 | 7.10 |
| 500 | 4.20 |
| 1000 | 2.90 |
| 2000 | 1.40 |

2) Did you work up the remaining concentrations of [NAD] from last week's reactions at 25uM NAM? Did they all show < 5% inhibition at 30 mins? You can post all the data from last 2 weeks to wiki in addition to answering this question.

AU: I have data for 50, 1000, 3000 uM NAD with 25 uM NAM for 30 min. I will post them on wiki.

3) Are the results with FdL2 peptide from today's report consistent with those from Guan using in-house enzyme? This would confirm that Enzo results can be extended to in-house in these studies.

AU: In-house Sirt3, 3000 uM NAD, 250 uM FdL peptide, 30 min, NAM 25 uM, in 5% DMSO

% activity remaining: 40.8% using Tecan.

AU data: 5U Enzo Sirt3, 3000 uM NAD, 250 uM FdL peptide, 30 min, NAM 50 uM, in 5% DMSO

|  |  |
| --- | --- |
|  |  |
| [NAM], uM | [P], uM, FdL | % activity |
| 0 | 2.376 | 100 |
| 50 | 1.216 | 51.17845 |

4) These results also suggest that 100uM NAM used by Thomas did not significantly slow the reaction.

How do the reaction conditions from today's report compare to those used by Thomas in our MST in presence of 100uM NAM? It seems you are only seeing very small % conversion (around 2%) of peptide substrate into product in 30 mins at saturating concentrations of both substrates?

AU: As per SM the MST buffer-

|  |  |  |
| --- | --- | --- |
|   | MST Exp | AU-HPLC |
| Tris-HCl pH 8.0 | 47 mM | 50 mM |
| NaCl | 129 mM | 137 mM |
| KCl | 2.5 mM | 2.7 mM |
| MgCl2 | 0.94 mM | 1 mM |
| DMSO | 5% | 5% |
| Tween-20 | 0.05% | No Tween |

Amount of product produce in different concentrations of NAM in 30 min when both the substrates are saturating is shown below-

|  |
| --- |
| 5% DMSO, 3000 um NAD, 600 uM K122, 30 min, 5U/rxn Enzo Sirt3 |
| [NAM], uM | % Product produced |
| **0** | 2.88 |
| **25** | 2.75 |
| **50** | 2.44 |
| **100** | 2.02 |
| **200** | 1.64 |
| **500** | 1.03 |

5) You and Guan should choose a [NAM] that produces approximately the same % inhibition as 25uM in the case of FdL2 peptide and use that for the planned initial rate experiments with MnSOD and nonzero NAM. Once you have the results in absence of honokiol and both 0,nonzero NAM for MnSOD, provide me with that mixed fitting.

AU: XG and AU will discuss and finalize the schedule.