LETTER

On the Nonspecific Degradation of NAD^+ to Nicotinamide Riboside

In a study aimed at identifying factors contributing to nicotinamide riboside (NmR) metabolism in yeast, Drs. Lu and Lin prepared a cellular extract by bead-beating in ice-cold 50 mM ammonium acetate (1). In Figs. 1–4, they added this extract to an NmR-dependent yeast strain and measured resulting growth. From the growth, they claimed to know either the relative or the absolute intracellular levels of NmR. The assay did not allow the authors to see that a yeast strain deleted for Isn1 and Sdt1, which has only 0–18% of the wild-type amounts of NmR (2), has reduced NmR levels. However, the extraction method would preserve the activity of virtually all enzymes, including those released from membranes and organelles. As such, the NmR-dependent growth assay might be reporting on all metabolites, including nicotinamide mononucleotide (NMN) and NAD^+, that are degraded to NmR in the extract. LC-MS analysis (Fig. 3) proves this to be the case. By dividing the authors' metabolite levels (in attomoles) by intracellular volume (70 fl), it is apparent that cellular NAD^+ (<0.01 mM) had degraded to NmR (>1 mM). When metabolites are preserved by rapidly boiling cells in buffered ethanol, the concentrations are roughly the reverse (3) of those reported by Lu and Lin. Moreover, in the course of making the principle claim of the paper, that the vacuolar phosphatase Pho8 encodes a "major NmR production factor," data in Fig. 3 establish that Pho8 is active in degrading NMN to NmR long after the fact of extracting metabolites from cells.

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DOI 10.1074/jbc.L110.217885

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