Direct determination of kinase activity using real-time ^{31}P NMR

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Using ^{31}P NMR, we have developed an assay to determine the activity of kinases and other enzymes that catalyse phosphoryl transfer. By running the reaction directly in a NMR tube, the method provides a non-intrusive way of determining the substrate and product concentrations during the course of the studied reaction.

This method eliminates the need for coupling enzymes regularly used to determine the activity of kinases. Such coupling enzymes introduces an extra layer of complexity, especially in studies of enzymes during stress conditions or extremophile enzymes with optimal activity in conditions very different to those of the most commonly used coupling enzymes. Although being a powerful way to quantify enzymatic catalysis directly and in real-time, the assay has some limitations that are dependent on the physics of the NMR methodology and some of these limitations is discussed in the poster.

We have demonstrated the usefulness of the ^{31}P NMR method in a number of studies, both published and yet unpublished. For example, we have quantified the activity of kinases in different chemical environments (1, 2) determined mechanisms of enzymatic selectivity by using custom synthesized and novel substrates (3-5) and determining the temperature dependency of thermophilic and phsycorphilic enzymes. A variant of the assay has also been used to determine external ATP degradation by pathogenic *E. coli*. In summary the assay has been found to be extremely useful by focusing on ^{31}P and can easily be extended also to other nuclei (such as ^{19}F). We are fully open to discuss new applications towards other systems.

References

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