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This Week's Citation Classic®

Glynn I M & Chappell J B. A simple method for the preparation of ^{32}P-labelled adenosine triphosphate of high specific activity. *Biochemical J.* 90:147-9, 1964.
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A method is described for preparing ^{32}P-labelled adenosine triphosphate (ATP) of high specific activity, by a simple process that requires only commercially available substrates and enzymes. Hydrolysis of the labelled ATP with heavy meromyosin shows that 98-99 percent of the ^{32}P is in the γ phosphate. [The *SCI*® indicates that this paper has been cited in over 1,970 publications.]

A Recipe for Hot ATP

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By the early 1960s, work in Cambridge and at Vanderbilt had shown that the sodium pump in cell membranes was identical with the $(Na^+ + K^+)$-activated ATPase that J.C. Skou had detected in membrane fragments. The complexity of the overall reaction catalysed by the pump suggested that the reaction involved a sequence of steps. One way of trying to elucidate this sequence was to allow the pump to hydrolyse [γ-^{32}P]ATP and to see where the radioactivity went. At that time ^{32}P-labelled ATP of high specific activity was not available commercially, and none of the reported methods for preparing it, whether chemical or enzymic, was very satisfactory. We therefore decided to develop a method that was simple, that gave a good yield, and that required only commercially available enzymes and substrates.

The method we finally adopted made use of the exchange reaction between [^{32}P]orthophosphate and the terminal phosphate of ATP that occurs in the presence of phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, and 3-phosphoglycerate. If the ingredients are mixed and allowed to stand for an hour at room temperature, more than 80 percent of the radioactivity is incorporated into the ATP; of this incorporated radioactivity, more than 98 percent is in the terminal phosphate. If carrier-free [^{32}P]orthophosphate is used, the specific activity of the labelled ATP can be extremely high. Radiation damage to the enzymes does not seem to be a problem. On one occasion, to the dismay of the laboratory radiation officer, we used 100 mC of ^{32}P. The method worked as usual, though the intensity of radiation was sufficient to darken the glass vessel in which the labelled ATP stood overnight.

In the innocent England of a quarter of a century ago, it seemed selfish to patent a method developed in a university laboratory and likely to be of value to colleagues involved in medical research. So we made no attempt to do so. But as well as publishing the method, we also wrote to the Radiochemical Centre, at Amersham, suggesting that they might like to use it. Their response was less than enthusiastic—they were not set up, they said, to use enzymes. Only after we had pointed out that all the ingredients could be bought in bottles, like groceries, and that no special enzymological skills were required, did they agree to try; and it was not until more than a year later, if I remember correctly, that they first marketed the product. Nowadays, despite the ready availability of [γ-^{32}P]ATP from commercial sources, our method is still widely used in individual laboratories, not only for making [γ-^{32}P]ATP itself,[1] but also for making γ-^{32}P-labelled ATP derivatives and analogues[2,3] and other γ-^{32}P-labelled nucleotides.[4]

1. **Penefsky H S.** Rate of chase-promoted hydrolysis of ATP in the high affinity catalytic site of beef heart mitochondrial ATPase. *J. Biol. Chem.* 263:6020-2, 1988.
2. **Gilles A-M, Marlière P, Rose T, Sarfati R, Longin R, Meier A, Fermandjian S, Monnot M, Cohen G N & Bârzu O.** Conservative replacement of methionine by norleucine in *Escherichia coli* adenylate kinase. *J. Biol. Chem.* 263:8204-9, 1988.
3. **Tesi C, Travers F & Barman T.** Transient kinetics of the interaction of 1, N^6-ethenoadenosine 5′-triphosphate with myosin subfragment 1 under normal and cryoenzymic conditions: a comparison with adenosine 5′-triphosphate. *Biochemistry* 27:4903-8, 1988.
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