Article title: Anomalies, contradictory data and dishonest scientific report in the paper entitled "Phosphorylation of DARPP-32, a dopamine and cAMP regulated phosphoprotein by casein kinase II", authored by Girault, J.-A., Hemmings, H.C.Jr., Williams, K.R., Nairn, A. and Greengard, P. (1989) and published in the Journal of Biological Chemistry [J. Biol. Chem. (1989) Vol. 264, pp21748-21759].

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Abstract.

The paper entitled "Phosphorylation of DARPP-32, a dopamine and cAMP regulated phosphoprotein by casein kinase II", authored by Girault, J.-A., Hemmings, H.C.Jr., Williams, K.R., Nairn, A. and Greengard, P. (1989) and published in the Journal of Biological Chemistry [J. Biol. Chem. (1989) Vol. 264, pp21748-21759] stated that DARPP-32 was phosphorylated by Casein kinase II on serines 45 and 102, that these sites were dephosphorylated by the catalytic subunits of protein phosphatase-1 (PP-1) and protein phosphatase-2A (PP-2A) and that the dephosphorylation of serines 45 and 102 of DARPP-32 by PP-1 and PP-2A was inhibited by prior phosphorylation of DARPP-32 on threonine 34 by PKA. These studies are problematic because the claim that the authors prepared and used PP-1 and PP-2A catalytic subunits as described in [2] was false based on their specific activities. The authors cannot explain their stated specific activities of the catalytic subunits of PP-1 and PP-2A that were so different from the published paper that they claimed to have followed to obtain their enzymes. The conclusion that serines 45 and 102 were preferentially dephosphorylated by PP-2A is therefore misleading at best and dishonest at worse.

The authors of the paper entitled " Phosphorylation of DARPP-32, a dopamine and cAMP regulated phosphoprotein by casein kinase II" that was published in the Journal of Biological Chemistry [J. Biol. Chem. (1989) Vol. 264, pp21748-21759] stated that they have shown that (i) DARPP-32 was phosphorylated by Casein kinase II (CKII) on serines 45 and 102, (ii) phosphorylation of DARPP-32 by CKII renders DARPP-32 a better substrate for phosphorylation on threonine 34 by PKA but not by cGMP, (iii) serines 45 and 102 were preferentially dephosphorylated by PP-2A and to a lesser extent by PP-2B and PP-1 and (iv) the dephosphorylation of CKII sites by PP-1, PP-2A and PP-2B were all inhibited by prior phosphorylation of DARPP-32 on threonine 34 by PKA.

The finding that the dephosphorylation of serines 45 and 102 by PP-2A and PP-2B is inhibited by prior phosphorylation of DARPP-32 on threonine 34 by PKA was not only problematic but contradicted previous published results [1], thereby questioning the specificity of the phosphorylation dependent inhibition of PP-1 by DARPP-32 phosphorylated on threonine 34 by PKA. The authors claimed that they purified PP-1 and PP-2A catalytic subunits according to published procedure by Cohen and co-workers [2]. However, careful examination showed that they did not purify PP-1 and PP-2A catalytic subunits as described in [2]. The authors stated in Table IV that the specific activities of the catalytic subunits of PP-1, PP-2A, and PP-2B were 0.16 μmol/min/mg, (i.e 160 nmol/ min/mg or 160 unit/mg), 0.66 μmol/min/mg (i.e 660 nmol/min/mg or 660 unit/mg), and 0.11 mol/min/mg (i.e 110 nmol/min/mg or 110 unit/mg) respectively. As noted in [3], it quite laughable and impossible for $PP\text{-}1_C$ and $PP\text{-}2A_C$ to have specific activities of 160 units/mg and 660 units/mg respectively using ^{32}P-labeled phosphorylase a as substrate [See the Scientific Articles, Characterization of a Reconstituted Mg-ATP-Dependent Protein Phosphatase by Therese J. Resink et al., Eur. J. Biochem. (1983) Vol. 133 pp455-461, The Catalytic Subunits of Protein Phosphatase-1 and Protein Phosphatase-2A are Distinct Gene Products by H.Y.L. Tung et al., Eur. J. Biochem. (1984) Vol 138, pp635-641, and Isolation and Characterization of Rabbit Skeletal Muscle Protein Phosphatases C-I and C-11" by Steven R. Silberman et al., J. Biol. Chem. (1984) Vol. 259, pp 2913-2922.] [4-6]. The above shows that either the authors did not purify the catalytic subunits

of PP-1 and PP-2A as they claimed or the data relating to the specific activities of PP-1_C and PP-$2A_C$ were fabricated and/or falsified.

The finding that the dephosphorylation of serines 45 and 102 of DARPP-32 by PP-2A could be inhibited by almost 70% by DARPP-32 phosphorylated on threonine 34 indicated that the preparation of PP-2_C was not pure and was contaminated by PP-1_C or the data was fabricated or falsified. The finding that the dephosphorylation of serines 45 and 102 of DARPP-32 by PP-2B could be inhibited by almost 78% by DARPP-32 phosphorylated on threonine 34 indicated that the preparation of PP-2B was not pure and was contaminated by PP-1_C or the data was fabricated or falsified. In view of the fact that the specific activity of PP-1_C reported in the paper by Girault et al. [7] is off by almost ~150 to 250 fold (~160 units/mg as opposed to ~20,000 to 40,000 units/mg) (Compare the specific activities of PP-1_C in published papers [4-6]), the results described in Table IV of their paper was at best misleading and dishonest at worse.

It is axiomatic that under normal circumstances, before using an enzyme that one had purified according to a published procedure and that had a very low specific activity compared to the published procedure and other procedure published by a different group of workers, one would first determine why the enzyme preparation had a low specific before using it in further studies. Because the enzyme used in the studies by Girault et al. [7] is not what it was described to be, the results that emanated from the use of the enzyme is problematic for a number of reasons. The enzyme had a low specific activity because it could be impure so that any reaction ascribed to it is misleading at best and dishonest at worse. The enzyme had a low specific activity because it was not pure and contained inhibitory molecules which would distort any inhibitory enzyme assays that the authors were performing [7].

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