Deamination of Adenosine Diphosphate by Adenylate Deaminase Preparations

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The deamination of adenine nucleotides by muscle preparations is generally assumed to proceed via adenosine 5'-monophosphate (AMP) through the action of a specific adenylate deaminase associated with myosin. In recent reports, evidence was presented for an additional route of deamination: direct deamination of adenosine diphosphate (ADP) by actomyosin gel and washed myofibrils. Deamination of ADP can likewise be effected by preparations of adenylate deaminase obtained from water extracts of skeletal muscle by the method of Kalckar. Whereas only AMP is deaminated under the usual conditions of the adenylate deaminase test (1–5 μg protein/ml and a reaction time of up to 60 min.), higher protein concentrations and longer reaction times lead to the deamination of ADP as well. 80–90% deamination was observed in reaction mixtures containing 5 mg protein and 7 μmoles ADP per ml in 0.1 M succinate buffer, pH 6.0 at 30°C and using a reaction time of 4 hours. Ion exchange and two-dimensional paper chromatography were used for analysis of the deproteinized reaction mixtures. Inosine diphosphate (IDP) was found to be the main product of the deamination of ADP and was isolated as the barium salt from pooled fractions of the effluent from ion exchange columns and identified by chemical analysis (hypoxanthine: ribose: acid labile P: total P). Beside IDP, minor amounts of inosine 5'-monophosphate (IMP) were detected and a further inosine nucleotide fraction, which preceded IDP in the ion exchange effluent and contains hypoxanthine, pentose, acid labile and acid stable phosphate in the proportions 2:2:1:2, corresponding thus in its composition to an equimolar mixture of IMP and IDP, or to a diinosine triphosphate.

Experiments on the pH-dependence of ADP deamination by adenylate deaminase preparations point to a pH optimum of near pH 6, in harmony with previous findings on actomyosin gel.

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On the Deaminase Component of Actomyosin

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It has been shown previously that dephosphorylation of adenosine triphosphate (ATP) by actomyosin is followed by deamination of the diphosphate initially formed, yielding inosine diphosphate (IDP) — or an IDP compound converted to IDP during isolation — as the final reaction product. At 20°C and pH 6.8–7.1 (without addition of buffer), using 5–25 mg (dry weight) actomyosin gel and 5 μmoles ATP per ml, dephosphorylation is complete within 30 minutes, whereas deamin-