Adenylate kinase from *Rhodospirillum rubrum*[1,2]

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Summary. A partial purification and some properties of adenylate kinase from the photosynthetic bacterium *Rhodospirillum rubrum* are described.

Adenylate kinases (EC 2.7.4.3.) from microorganisms were first investigated in 1956 by Oliver and Peel[4]. The authors reported finding adenylate kinase activity in 15 organisms *Rhodospirillum rubrum*, but no attempt was made to characterize the enzymes. Since then, adenylate kinases from several microorganisms have been isolated and characterized[5-8]. Adenylate kinase has not been isolated from a photosynthetic bacterium, however. Our interest in adenylate kinase from *R. rubrum* resulted from our research on the photosynthetic regeneration of ATP from AMP using bacterial chromatophores[9,10]. We reasoned that isolation and use of adenylate kinase and chromatophores from the same organism would be an economic use of cells. Adenylate kinase activity was assayed by measuring the rate of ATP formation from ADP in a coupled enzyme system, essentially as described by Oliver[11]. Protein was determined by the method of Lowry et al.[12], or by the method of Warburg and Christian[13] for column fractions.

Rhodospirillum rubrum (ATCC 11170) was grown anaerobically as described by Pace et al.[9]. 30 g (wet weight) of cells were suspended in 60 ml 0.05 M Tris buffer, pH 7.8, and disrupted using a Braun rotary homogenizer (Bronwill Scientific Inc., Rochester, N.Y.) at 5-10 °C for 3 min. The broken cell suspension was centrifuged at 19,000× g for 30 min. The supernatant was recentrifuged at 100,000× g for 60 min and the high speed supernatant was designated as the crude extract (table).

After addition of 1 mM dithiothreitol (DTT), the pH of the crude extract was slowly lowered to 3.5 by addition of 1 M citric acid at ice bath temperature. The solution was allowed to stir for 5 min, then the pH was raised to 7.5 with 1 N NaOH. The precipitate was centrifuged at 12,000× g for 15 min. Although there is only a small increase in specific activity following the acid precipitation, the step is included because it helps to eliminate red pigment present in the crude extract. Ammonium sulfate was next added to the supernatant to 30% saturation. The precipitate was centrifuged (12,000× g for 15 min), and the supernatant was brought to 75% saturation and recentrifuged (12,000× g, 15 min). The pellet was redissolved in 1-2 ml buffer, applied directly to a column of Sephadex G-75 (1.5×90 cm) and eluted with 0.1 M Tris buffer, pH 7.8. 4.5 ml fractions were collected at a flow rate of 20 ml/h. Approximately 85% of the protein applied to the column eluted in 20-30 ml after the void volume. Most of the adenylate kinase activity eluted between 58 and 77 ml. The fractions having the highest activity were combined and applied to a column of DEAE-cellulose (1.5×18 cm). The ion exchange column was equilibrated with 0.05 M Tris buffer, pH 7.8, and after non-adsorbed proteins were washed out (45 ml), the enzyme was eluted using a linear gradient prepared by mixing 150 ml of 0.05 M Tris buffer, pH 6.0, with 150 ml of 0.5 M Tris buffer, pH 6.0. 4.5 ml fractions were collected at a flow rate of 18 ml/h. Most of the adenylate kinase activity eluted between 121 and 144 ml. The fractions having the highest activity were combined to yield a preparation with a specific activity of 6.4 units/mg protein.

Purification of adenylate kinase from *Rhodospirillum rubrum*

Fraction	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purification
Crude extract	61.7	560	0.11	100	1.0
Acid precipitation	69.3	533	0.13	100	1.2
Ammonium sulfate precipitation	48.9	200	0.24	79	2.2
G-75 gel	23.5	38	0.62	38	5.6
DEAE-cellulose	7.3	1.1	6.39	12	58

Polyacrylamide gel electrophoresis of this sample according to the method of Gabriel[14] (System I, 7.5% acrylamide) revealed 2 minor bands in addition to the major band. The latter was estimated to represent 90% of the total protein applied to the gel. The purified enzyme sample was also applied to an isoelectric focusing column (LKB8100, LKB Produkter, Bromma, Sweden), 110 ml capacity, containing an ampholyte solution with a pH range of 3-10. The current was maintained at 5 mA for 24 h. 3 ml fractions were collected and assayed for enzyme activity. The enzyme focused as a single band, and its isoelectric point (pI) was found to be 5.0. This compares to pI values for adenylate kinase from *Thiobacillus neapolitanus* of 4.2[6], and *Escherichia coli* of 5.0[7]. Mammalian adenylate kinase isozymes have isoelectric points varying from 4.7 to >11[15,16].

Adenylate kinase from *R. rubrum* is an extremely labile enzyme. Its stability depends on the presence of reducing agents. At 3 °C, the half life of the enzyme was 55 h in the presence of DTT, and 35 h in the absence of DTT. In the frozen state, in the presence of DTT, 85% of the activity remained after 72 h and 44% after 84 days.

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