INTERACTIONS OF FLUORESCENT ANALOGS OF ADENINE NUCLEOTIDES WITH COUPLING FACTOR PROTEIN ISOLATED FROM SPINACH CHLOROPLASTS

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Received 7 July 1975

1. Introduction

The coupling factor protein (CF_1) of photosynthetic membranes plays a central role in the process of energy transduction [1–3]. We have studied the interaction of this enzyme with its proposed substrates (i.e., AMP, ADP and ATP; ref. [4,5]) by using their fluorescent analogs ϵAMP, ϵADP and ϵATP [6]). Some spectroscopic properties of these fluorophores and their biological activities (with other enzymes) have been described [7–10]. Moreover, it has been demonstrated that ϵADP acts nearly as well as ADP as substrate for photophosphorylation (11; unpublished observations of the authors). We report here the first use of the fluorescence properties of the ϵ-adenine analogs in studying their interaction with the CF_1 enzyme isolated from spinach chloroplasts. We have shown that ϵADP and ϵATP, but not ϵAMP, bind to purified CF_1, that differences in binding exist depending on whether the associated divalent cation is Mg^{2+} or Ca^{2+}, and that orthophosphate reduces the level of Mg^{2+}-dependent binding of ϵADP to CF_1.

2. Materials and methods

Pure coupling factor protein (CF_1) was isolated from spinach leaves according to the method of Strotmann et al. [12] and concentrated by ammonium sulfate precipitation. CF_1 was stored at 4°C in the presence of 25 mM Tricine–NaOH (pH 8), 4 mM ATP, 2 mM EDTA and 2 M $(NH_4)_2SO_4$; this medium is known to maintain the stability of CF_1 for several months [13]. For use in fluorescence experiments, aliquots of stored CF_1 were centrifuged, dissolved in a minimal volume of 25 mM Tricine buffer (pH 8) and then desalted on a column (4 cm × 17 cm) of Sephadex G-200. Protein concentrations were determined by the Lowry method [14]. A molecular weight of 325 000 for CF_1, as found by Farron [15], was used in determining its molarity.

The fluorescent nucleotides $1,N^6$-ethenoadenosine mono-, di- and triphosphate (ϵAMP, ϵADP and ϵATP) were purchased from P & L Biochemicals, Inc., Milwaukee, Wis. and Tricine from Sigma, St. Louis, Mo. Fluorescence polarization measurements were made on a photon counting instrument in Professor. G. Weber's laboratory [16]. This apparatus allowed the convenient subtraction of background interference at low signal-to-noise values. A 450-Watt xenon arc, cooled by air draft, was the light source. The 310 nm exciting light was selected by a Bausch and Lomb grating monochromator and passed through a Corning glass CS 7-54 filter. The cuvette holder fits square cuvettes of 1 cm internal dimensions. The fluorescence emission was filtered through Corning glass CS 0-52 and CS 3-75 filters.

Values of I// and I⊥, where I// is the intensity of the emission light polarized parallel to the exciting beam and I⊥ is the intensity of the emission light polarized perpendicular to the exciting beam, were obtained by counting over one-half minute intervals. All fluorescence measurements were made at room temperature, since CF_1 in solution is cold labile [13].

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3. Results and discussion

If the relatively small fluorescent nucleotides (mol. wt. $\leqslant 600$) bind to the relatively large (mol. wt. = 325 000), slowly rotating CF_1 macromolecule, the polarization of fluorescence (p) is expected to increase above the $p = 0$ level for the unbound fluorophore. Fluorescence polarization values significantly above the zero level were observed when ϵADP or ϵATP, but not ϵAMP, was added to a solution of CF_1 (figs. 1 and 2). As the concentration of added ligand (ϵADP or ϵATP) is increased above nearly equimolar CF_1: ligand, polarization decreases toward $p = 0$ as a greater percentage of the total fluorescing population remains unbound. We also note that binding with ϵADP appears greater than with ϵATP.

A distinct difference in the binding curve is observed, depending on whether Mg^{2+} or Ca^{2+} is present as the divalent cation (fig.1). This difference probably reflects the existence of separate ion-dependent conformers of CF_1 and/or distinct modes of binding to the protein. This data could be at least qualitatively related to previous observations of Mg^{2+} vs Ca^{2+} differences in CF_1 activities [2, 17–19]. (However, see refs. [12, 20–22] where 'allotopic' properties towards divalent cation have been questioned.)

Fig.2 shows the effect of orthophosphate on the Mg^{2+}-dependent binding of ϵADP and ϵATP to CF_1. While there appears to be little or no effect of phosphate on the ϵATP binding, the presence of phosphate significantly reduced the level of ϵADP binding to CF_1 as indicated by fluorescence polarization. In experiments with the Mg^{2+}-dependent ATPase (in chloroplasts), Carmeli and Lifshitz [23] found that orthophosphate prevented the destabilization of ATPase activity as induced by ADP. Our results in fig.2 suggest that this is due to the interference by phosphate of ADP binding to CF_1, either because of a phosphate induced change in the conformational state of CF_1 or a direct competition at the ADP binding site.

In conclusion, we have established (1) that there is binding of the nucleotides ϵADP and ϵATP with isolated

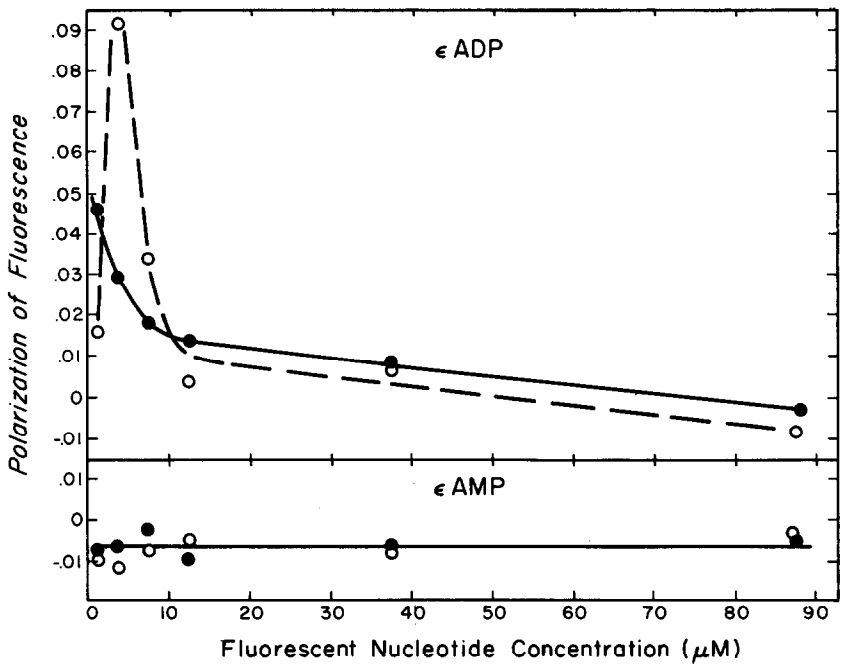

Fig.1. Degree of polarization of ϵ-adenine analog fluorescence as a function of concentration of adenine analogs. CF_1 protein ($\sim 4 \times 10^{-6}$ M) was buffered at pH 8 in 25 mM Tricine–NaOH, plus 5 mM $CaCl_2$ (open circles) or 5 mM $MgCl_2$ (closed circles). Instrument fluctuations introduce an error of approximately ± 0.002 in these values of p. See Materials and methods for other experimental details.

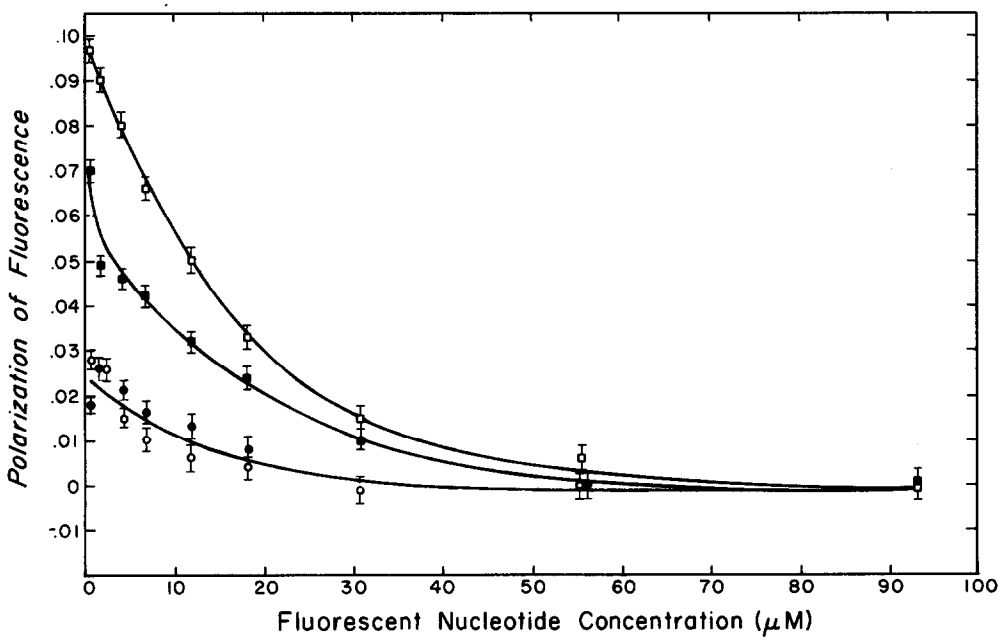

Fig.2. Degree of polarization of ϵ-adenine fluorescence as a function of concentration of adenine analogs. CF_1 protein was buffered at pH 8 in 25 mM Tricine–NaOH, plus 5 mM $MgCl_2$ and 500 μM NaH_2PO_4 (closed symbols) or 5 mM $MgCl_2$ only (open symbols). [CF_1] was ~4 x 10^{-6} M (ϵATP data, circles) and ~5 x 10^{-6} M (ϵADP data, square). Error bars represent approximate contribution of instrument fluctuation.

CF_1, with no binding for ϵAMP; (2) differences in the binding characteristics when Mg^{2+} or Ca^{2+} is present; and (3) inorganic phosphate decreases the Mg^{2+}-dependent binding of ϵADP, but not ϵATP. As a working hypothesis, we suggest that the structure of CF_1 is such that (a) there are different sites and/or modes of binding for Ca^{2+} and Mg^{2+}; (b) the ATP binding site covers the binding sites for both ADP and P_i allowing no effect of P_i on ATP binding; (c) *if* AMP is actually involved in the terminal steps of photophosphorylation [5], it must be able to bind to CF_1 only as the protein's conformational state becomes altered (e.g., upon energization by light); and (d) inhibition of ADP binding by P_i can be explained on the basis of a change in conformation of CF_1. These hypotheses, however, need to be tested.

Acknowledgements

This research was supported in part by a predoctoral traineeship from the NIH (HEW PHS GM 00720).

We are grateful to Dr. Jorge R. Barrio and Professor. Nelson J. Leonard for advice and for samples of fluorescent nucleotides used in a preliminary phase of this work. We especially thank Dave Jameson for helpful discussions and Professor Gregorio Weber for the use of polarization instrument.

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