PHOSPHOLIPID-INDUCED CHANGES IN THE CIRCULAR DICHROISM OF GLYCOPHORIN AND ITS ASSOCIATION WITH 8-ANILINO-1-NAPHTHALENE SULFONATE

J. A. VERPOORTE and F. B. St. C. PALMER
Department of Biochemistry, Dalhousie University, Halifax, NS, B3H 4H7, Canada

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1. Introduction

Previous studies have shown that polyphosphoinositides represent most of the metabolically active phosphorus that is bound to erythrocyte membrane protein in a proteolipid complex [1,2]. Glycophorin is the principal intrinsic sialoglycoprotein of human erythrocyte membranes [3]. Acidic phospholipids, particularly phosphatidylserine [4] and diphosphoinositide [5] remain bound to glycophorin during isolation by the diiodosalicylate method [6]. Therefore, certain acidic phospholipids may be specifically associated with glycophorin in the membrane, although non-specific binding can not be excluded.

In this communication we report the interaction of glycophorin with various phospholipids as monitored by circular dichroism (CD) and the fluorescence of 8-anilino-1-naphthalene sulfonate (ANS). Di- and triphosphoinositides or lysophospholipids increased the ellipticity (θ_{220}) of both native and sialic acid-free glycophorin. The polyphosphoinositides also decreased the fluorescence of ANS–glycophorin complexes. Other diacyl phospolipids had little or no effect.

2. Materials and methods

Chromatographically purified glycophorin was prepared from human erythrocytes (type 0^+) after extraction of the membranes with *n*-butanol, at pH 8.0 [7]. Concentrations of glycophorin were calculated from the sialic acid content [8]. Sialic acid-free glycophorin was prepared as described [8], and its concentration determined from the absorbance at 280 nm which was compared to that of native glycophorin.

Triphosphoinositide was isolated from bovine brain [9]. Diphosphoinositide was prepared from triphosphoinositide using the soluble triphosphoinositide phosphatase from *Crithidia fasciculata* [10]. Phospholipids from natural sources and synthetic phospholipids obtained from Serdary Research Laboratories (London, Canada) were repurified by thin-layer chromatography [11]. Lipids were suspended in water by brief sonication, and aliquots of ca. 1 mM suspensions were added to buffered solutions of glycophorin. Phosphorus was determined by the method of Bartlett [12].

CD was measured with a Cary spectropolarimeter model 6001 [7], and fluorescence was measured with an Aminco-Bowman spectrofluorimeter using horizontally polarized light at 355 nm for excitation [8]. The emission of ANS (obtained as the ammonium salt from Pierce Chemical Co., Rockford, IL) was measured at 470 nm. All measurements were done at 25°C.

3. Results

3.1. *Circular dichroism*

The purified glycophorin contained only 0.03 ± 0.006% phosphorus, half of which could be extracted with acidified chloroform-methanol. These preparations were therefore essentially free of phospholipid. The addition of triphosphoinositide to aqueous solutions of glycophorin, or its sialic acid-free counterpart, produced changes in the CD spectra (fig.1). Within the limits of experimental error the

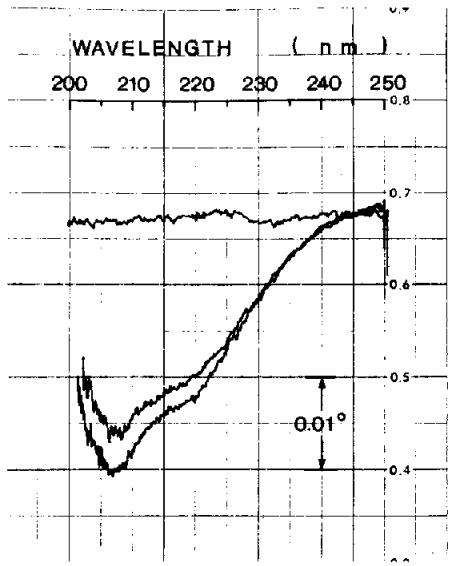

Fig.1. The effect of triphosphoinositide on the circular dichroism of glycophorin. The CD spectra of 3.7 μM glycophorin in 0.01 M Tris–chloride, pH 7.0, was measured in a 1 cm cell (upper curve) and again after addition of 28 μM triphosphoinositide (lower curve).

increase in θ_{220} appeared to be a function of the triphosphoinositide/glycophorin molar ratio. However, the incremental increase became small at ratios above ca. 6.0. The effect of triphosphoinositide was independent of pH between 3.0 and 7.5 but was slightly enhanced by increasing the ionic strenght with NaCl. Diphosphoinositide and lysophospholipids produced similar changes in the CD of glycophorin and sialic acid-free glycophorin. Other diacyl phospholipids had no effect, except yeast phosphatidylinositol which caused a small increase in θ_{220} at higher concentrations (table 1). Inositol di- and triphosphates prepared enzymically from di- and triphosphoinositides [10] also had no effect.

Triphosphoinositide also increased the ellipticity when added to mixtures of glycophorin and phosphatidylserine or liver phosphatidylinositol. Only yeast phosphatidylinositol reduced this effect of triphosphoinositide. The same results were obtained with phospholipids of natural origin and with synthetic phospholipids containing palmitic acid.

Table 1
The effect of phospholipids on the CD spectrum of glycophorin[a]

Phospholipid	Molar ratio lipid/glycophorin	Percent change in θ_{220} [b] A	B
phosphatidylcholine[c,d]	10	NC	NC
lysophosphatidylcholine[c,d]	9	+11.4	+12.2
phosphatidylethanolamine[c,d]	10	NC	NC
phosphatidylserine[e]	10	NC	NC
phosphatidic acid[c,f]	9	NC	NC
lysophosphatidic acid[c]	10	+12.2	+13.9
phosphatidylinositol[d]	10	NC	NC
phosphatidylinositol[g]	16	+ 5.0	NC
diphosphoinositide[h]	6	+14.3	+13.2
triphosphoinositide[h]	6	+11.7	+13.1

[a]CD measurements made with 10 μM glycophorin in 10 mM tris-chloride buffer, pH 7.0
[b](A) native glycophorin; B sialic acid-free glycophorin; NC, no change (less than 3%)
[c]Synthetic palmitoyl phospholipid
[d]Pig liver
[e]Pig brain
[f]Prepared from egg lecithin
[g]Yeast
[h]Bovine brain

3.2. *Fluorescence of ANS*

The fluorescence of ANS–glycophorin mixtures was diminished by both di- and triphosphoinositides. Other diacyl phospholipids produced no changes, but lysophosphatidic acid reduced the fluorescence while lysophosphatidylcholine increased it. Double reciprocal plots of fluorescence intensity at a constant ANS concentration against increasing amounts of glycophorin mixed with phospholipids were examined (fig.2). The lines for all phospholipids gave the same intercept suggesting that the quantum yield of the bound dye remains unchanged in the presence of phospholipid. Polyphosphoinositides and lysophospholipids therefore must alter the apparent association constant of ANS with glycophorin. The results were similar with sialic acid-free glycophorin using either naturally-occurring or synthetic phospholipids.

4. Discussion

Glycophorin in aqueous solution exhibits specific affinity for lysophospholipids and polyphosphoinositides as demonstrated by CD. The increase in θ_{220} implies a conformational change in the protein, i.e., increased α-helix content. The sialic acid residues of glycophorin do not participate in this interaction with phospholipids nor are they involved in the conformational change. The same phospholipids interfere with the binding of ANS to glycophorin.

It has been reported that unsaturated phosphatidylcholines [13] and saturated phosphatidylserines [4] are preferentially bound to glycophorin. Since the results obtained with palmitoyl phospholipids and unsaturated natural phospholipids were similar, the degree of saturation is not very important for the conformational change in glycophorin. Although phosphatidylserine and other phospholipids interact with glycophorin [4,13], they do not change the conformation or the binding of ANS and they also do not prevent the binding of polyphosphoinositides. Thus, the changes in CD and ANS binding probably must be attributed to the nature of the hydrophillic moiety of the polyphosphoinositides. Since phosphatidylinositol and phosphatidic acid were also not effective, the interaction of the polyphosphoinositides with glycophorin must depend upon their greater negative charge and/or the location of these charges on the inositol ring at a greater distance from the hydrophobic portion of the molecule.

The polyphosphoinositides and the phosphatidylserines are located on the inner surface of the erythrocyte membrane [14,15]. Presumably, they bind to the positively-charged octapeptide segment of glycophorin which is also on the inner surface of the membrane [3,4]. The present study suggests that the phosphorylation of membrane bound phosphatidylinositol could result in an altered conformation of glycophorin. This finding may be of particular significance in view of the recent observation that a change in erythrocyte shape induced by a calcium ionophore is correlated with the loss of polyphosphoinositide from the membrane [16].

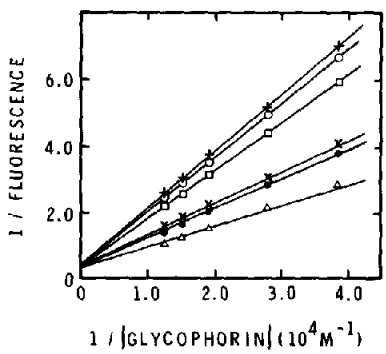

Fig.2. Double reciprocal plots of ANS fluorescence versus glycophorin–phospholipid concentrations. Aliquots of 156 μM glycophorin mixed with phospholipid in 0.01 M Tris–chloride, pH 7.0, containing 20 μM ANS were added to 20 μM ANS in the same buffer. Ratios of phospholipid to glycophorin were constant. Standard quinine sulfate dihydrate (Regis Chemical Co., Morton Grove, IL) was used to correct for instrumental fluctuations. The glycophorin solutions contained: no lipid (•); 784 μM triphosphoinositide (+); 777 μM diphosphoinositide (○); 904 μM lysophosphatidic acid (□); 895 μM lysophosphatidylcholine (△); and 745 μM phosphatidylinositol (×).

Acknowledgements

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