Quebec Cooperative Study
of Friedreich's Ataxia

Protein Kinase Activity of Human Erythrocyte Membranes in Friedreich's Ataxia

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SUMMARY: *Proteins of human erythrocyte membranes of Friedrich's ataxia patients and controls were examined by SDS-polyacrylamide gel electrophoresis before and after reduction with β-mercaptoethanol. No difference could be detected in the composition of their state of aggregation. The protein kinase activity of human erythrocyte membranes of eleven Friedreich's ataxia patients and six controls was determined. No difference in their protein kinase activity could be detected. These results are discussed with respect to an involvement of a generalized membrane defect in Friedreich's ataxia.*

RÉSUMÉ: *Les protéines des membranes érythrocytaires humaines de patients ayant l'ataxie de Friedreich et de contrôles furent examinées par électrophorèse sur gel de polyacrylamide en SDS, avant et après une réduction avec le β-mercaptoethanol. Aucune différence ne fut détectée dans leur composition, ou leur état d'aggrégation. L'activité de la protéine kinase des membranes erythrocytaires humaines fut déterminée chez onze patients avec l'ataxie de Friedreich et six contrôles. Aucune différence ne fut détectée dans l'activitée de la protéine kinase. Ces résultats sont discutés en rapport avec la participation d'un défaut membranaire généralisé dans l'ataxie de Friedreich.*

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INTRODUCTION

Previous studies have provided some evidence for a generalized membrane defect in Friedreich's ataxia. Specifically, it was observed that the amount of the fatty acid linoleic acid (C18:2) in phospatidylcholine, isolated from human erythrocyte membranes, was decreased in Friedrich's ataxia patients (Huange et al, 1980, this symposium) and that the physical state of human erythrocytes membranes proteins was altered in Friedrich's ataxia patients (Butterfield) et al, 1979). The direction of the alteration is qualitatively consistent with the decrease in linoleic acid (C18:2).

We have sought to obtain additional evidence for a generalized membrane defect in Friedreich's ataxia. To that end we have examined the proteins of human erythrocyte membranes by SDS-polyacrylamide gel electrophoresis before and after a reduction with β-mercaptoethanol since previous studies have suggested an alteration of its composition (Shapcott et al, 1979, unpublished results; see Draper et al, 1979). The protein kinase activity of human erythrocyte membranes was also determined since in a number of hereditary diseases, in which a defect of the membranes was known, an increase or decrease of its activity was observed (Roses and Appel, 1973 and 1975; Roses et al, 1975; Hosey and Tao, 1976; Dzandu and Johnson, 1980).

MATERIALS AND METHODS

Materials

Eleven Friedreich's ataxia patients (six females and five males) and six controls (three females and three males), which had fasted overnight, were used for our studies. Patients and controls used in a given experiment were age and sex-matched. Blood was drawn by venipuncture in heparin. Reagents for polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories; (γ-^{32}P)-ATP of specific activity 5-9 mci per mole was obtained from New England Nuclear. Solutions of (γ-^{32}P)-ATP made were used within a period of two weeks and stored at -20°C.

Preparations of erythrocyte membranes

Erythrocyte membranes were prepared with fresh solutions cooled at 4°C. Ten milliliters of freshly drawn blood in heparin were centrifuged at 27,000 g for 20 minutes. After removal of the supernatant and buffy coat the erythrocytes were washed three times with a solution of 310 mosm sodium phosphate buffer, pH 7.4, and lysed with a solution of 5 mM sodium phosphate buffer, pH 8.0. The erythrocyte membranes were collected by centrifugation at 27,000 g for 15 minutes and washed until white with the lysis buffer. They were then washed twice with a solution of 50 mM CH_3COONa, 10 mM $MgCl_2$ and 0.6 mM EGTA buffer, pH 6.5 and suspended in this washing buffer at a protein concentration of 2-3 mg per milliliter.

Protein kinase assay

The protein kinase assay was done at room temperature under conditions similar to those of Roses and Appel (1975), using the following procedure: Aliquots of erythrocyte suspension containing 160 μg of protein were diluted to a volume of 150 μl with a

Abbreviations:
SDS: sodium dodecyl sulfate; EDTA: ethylene dinitrilo tetraacetic acid; EGTA: ethyleneglycol-bis (β-aminoethyl ether); N, N'-tetracetic acid.

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solution of 50 mM CH_3COONa, 10 mM $MgCl_2$ and 0.6 mM EGTA buffer, pH 6.5. The reaction of phosphorylation was then initiated with the addition of 50 μl of a solution of (γ-^{32}P)-ATP (30 nmoles/ml of assay buffer). After 30 seconds the reaction was stopped with the addition of 300 μl of a solution of 20% sucrose, 3% SDS, 1% β-mercaptoethanol, 0.005% bromophenol blue, 1 mM EDTA and 10 mM sodium phosphate buffer, pH 7.2. Aliquots of 220 μl were then submitted to a SDS-polyacrylamide gel electrophoresis according to the procedure of Weber and Osborn (1969). Gels were stained with Coomassie blue and destained. The radioactivity count of the proteins bands was then determined according to a procedure previously described (Wong and Roses, 1979).

Protein determination

Protein concentration was determined according to Lowry's assay (1951) using a solution of Na_2CO_3 made 3% in SDS. Aliquots of erythrocyte membranes suspension were solubilized with a 1% SDS solution before protein determination. Bovine serum albumin was used as the standard.

RESULTS

The proteins of human erythrocyte membranes of controls and Friedreich's ataxia patients were examined by SDS-polyacrylamide gel electrophoresis, after a reduction with β-mercaptoethanol. The results are shown in Figure 1 and indicate no difference in their composition.

The proteins of human erythrocyte membranes of controls and Friedreich's ataxia patients were also examined by SDS-polyacrylamide gel electrophoresis without reduction with β-mercaptoethanol, in order to investigate the possibility that the proteins in Friedreich's ataxia patients may be cross-linked via disulfide bonds. The results showed that none of the proteins in Friedreich's ataxia patients were cross-linked via disulfide bonds: the proteins bands which were observed in controls and Friedreich's ataxia patients were the same as those observed when the electrophoresis was done with the human erythrocyte membrane proteins reduced with β-mercaptoethanol (see Figure 1).

The protein kinase activity of human erythrocyte membranes of controls and Friedreich's ataxia patients was determined. The procedure used involved basically a brief incubation of the erythrocyte membranes with (γ-^{32}P)-ATP, separation of the proteins by SDS-polyacrylamide gel electrophoresis and determination of the amount of ^{32}P incorporated into the different proteins bands visualized with Coomassie blue.

The results are summarized in Table 1 and show that the protein kinase activity of human erythrocytes of controls and Friedreich's ataxia patients are identical within experimental error.

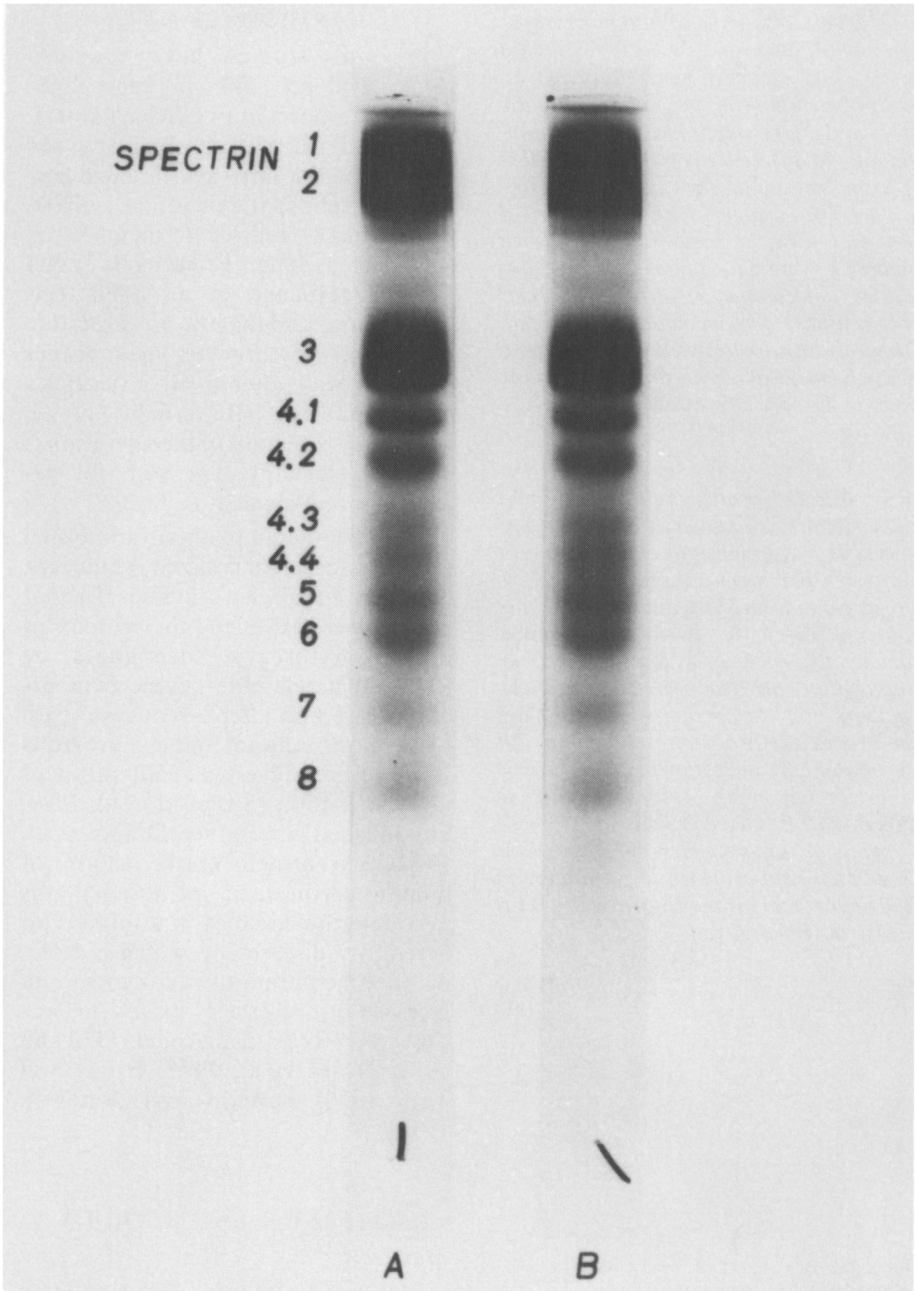

Figure 1 — Proteins of human erythrocyte membranes of control (Gel A) and Friedrich's ataxia patient (Gel B). Proteins were reduced with β-mercaptoethanol before electrophoresis. Proteins bands have been numbered according to a nomenclature of Fairbanks et al (1971).

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DISCUSSION

We have examined the proteins of human erythrocyte membranes by SDS-polyacrylamide gel electrophoresis before and after reduction with β-mercaptoethanol and have determined the protein kinase activity of human erythrocyte membranes in Friedreich's ataxia patients in order to obtain some additional evidence for a generalized membrane defect in Friedreich's ataxia patients. The results obtained show that the protein composition, the state of aggregation of the proteins and the protein kinase activity are normal in Friedreich's ataxia patients (Figure 1 and Table 1).

There are three conclusions which could be drawn from the above results and results previously reported: (1) There is no generalized membrane defect in Friedreich's ataxia. One has to contemplate this possibility since the alterations of human erythrocyte membranes reported (Draper et al, 1979 and Butterfield et al, 1979) are relatively minor. Other studies done with human erythrocytes have not indicated the presence of major membrane abnormalities (Bureau et al, 1978; Butterfield et al, 1979; Steinberg et al, 1979). (2) There is a generalized membrane defect in Friedreich's ataxia, but this is expressed only minimally in human erythrocyte membranes. The plasma membranes of different tissues are functionally and structurally different. Therefore one should not expect to have the same expression of a generalized membrane defect in all plasma membranes. (3) There is a generalized membrane defect in Friedreich's ataxia but it cannot be detected in human erythrocyte membranes because of the conditions used to prepare them. Conditions of preparation of human erythrocyte membranes have been shown to greatly affect their biochemical properties (Hanahan et al, 1973). Significantly, the alteration of the protein kinase activity in myotonic muscular dystrophy could only be observed under certain conditions of preparations of erythrocyte membranes (Roses and Appel, 1973; Roses and Appel, 1975).

The possibility of a generalized protein membrane defect in Friedreich's ataxia cannot be ruled out, although the present evidence leans to the contrary. Experiments are presently being carried out to investigate these aspects.

TABLE 1

Amount Of ^{32}P Incorporated Into The Proteins Bands Of Human Erythrocyte Membranes Of Controls And Friedreich's Ataxia Patients.

Protein Band[a]	^{32}P incorporated (pmoles/ml protein/30 sec.) Control	Friedreich's Ataxia
1 + 2 (spectrin)	7.23 ± 1.99 (6)[b]	7.33 ± 2.71 (11)
3	1.76 ± 0.64 (6)	1.76 ± 0.79 (11)
4.1 + 4.2	0.51 ± 0.15 (5)	0.59 ± 0.23 (10)
5 + 6	0.36 ± 0.09 (5)	0.43 ± 0.18 (10)
7	0.12 ± 0.05 (4)	0.17 ± 0.12 (7)
8	0.12 ± 0.05 (5)	0.13 ± 0.11 (10)

a) See figure 1 for the different proteins bands.
b) The number in parenthesis refers to the number of determinations. Each determination represents the average of two assays.

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