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INCREASED SULFATE UPTAKE IN SKIN FIBROBLASTS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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Sulfate uptake into skin fibroblasts from patients with cystic fibrosis is increased. Sulfate transport studies were carried out in skin fibroblasts isolated from age/sex matched cystic fibrosis and normal subjects. Sulfate transport occurred mainly via a carrier-mediated proton-stimulated SO_4^{2-}/Cl^- exchange. The capacity (V_{max}) of the uptake system operating at physiological concentrations of sulfate was stimulated in cystic fibrosis, but the affinity of the carrier for sulfate was not altered.

Cystic fibrosis (CF) is the most common autosomal-recessive disease of Caucasians, affecting one in every 1,600 to 2,500 live births (1). The pathophysiology of CF appears to be related to two major anomalies: (1) abnormal electrolyte composition of sweat and salivary glands manifesting itself as an increased salinity of the secretions, i.e., elevated Na^+ and Cl^-; (2) abnormal properties of the mucous secretions resulting in obstruction and frequently infection within the mucus-secreting organs (4,5).

Hypersecretion of mucus from tracheal explants of patients with CF does occur, and mucous glycoproteins are not secreted in preference to nonmucous glycoprotein components (4). The tracheobronchial secretions of patients with CF are more highly sulfated and exhibit stronger acidic properties than those from normals (6,7). An increased level of acid mucopolysaccharides has also been reported in cultured skin fibroblasts isolated from CF patients (8). There is evidence that solutions of highly sulfated mucins are more viscous than solutions of less sulfated mucins (9) and that there is a significant correlation between enhanced levels of highly sulfated mucins in tracheobronchial secretions of CF patients and increased disease severity (10). Despite the importance of altered macromolecular sulfation in CF,

[1]PAPS, adenosine 3'-phosphate 5'-phosphosulfate

[2]SITS, 4-isothiocyano-4'-acetamide stilbene-2,2'-disulfonic acid

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little is known about the factors which control this process. Evidence is available indicating that the universal sulfate-donor for macromolecular sulfation is PAPS[1] (11). Since the affinity of enzymes involved in PAPS synthesis for SO_4^{2-} is comparable to SO_4^{2-} levels in the serum (12) the intracellular levels of PAPS would be expected to be strongly affected by the size of the intracellular pool of inorganic sulfate. The potential sources for the intracellular pool of sulfate are inorganic sulfate and sulfate released by the intracellular oxidation of sulfur-containing amino acids.

In the present study we have investigated inorganic sulfate uptake in skin fibroblasts isolated from normal and CF patients. We have chosen to carry out our studies in skin fibroblasts because they are a readily available human tissue which may reflect the inherited biochemical abnormalities of CF. In fact, impaired chloride efflux (13), Ca^{2+} transport (14) and changes in glycoprotein composition (15) have been demonstrated in cultured human CF skin fibroblasts. Several previous studies have attracted our attention to the possibility that sulfate uptake may be affected in CF: (1) In many mammalian cells, sulfate uptake occurs via a SO_4^{2-}/Cl^- exchange mechanism (16,17,18,27); (2) Chloride permeability via a chloride channel has been shown to be markedly decreased in CF (19,20,21,22). We therefore postulated that altered sulfate uptake may occur concommittantly with the defect in chloride permeability. Our studies indicate that sulfate uptake is stimulated in skin fibroblasts isolated from CF patients.

METHODS

Human skin fibroblasts from age/sex-matched normal and cystic fibrosis subjects were obtained through the tissue culture core facility of the Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham. Fibroblasts were cultured from skin biopsies taken from the anterior aspect of the forearm as described by Sly and Grubb (23). Cultures were maintained at 37°C in a humidified environment of 5% CO_2 in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% heat inactivated fetal bovine serum. The fibroblasts were grown to confluence in 35 mm dishes (Falcon) in 3 ml medium. Normal and cystic fibrosis skin fibroblasts used for the experiments were in all cases subcultured for the same number of times (i.e. same passage number). Transport was measured in confluent cultures at 37°C, as previously described by us (18). Protein concentrations were determined by the method of Lowry (24). All transport experiments and assays were performed in duplicate or triplicate samples for each condition and results are generally given as a mean ± standard deviation (σ_{n-1}). The number of age/sex matched normal and CF cell lines used for each type of experiment and the total number of experiments of a certain type is given in each case with the results. The apparent Km and Vmax values obtained in kinetic studies were calculated using a computer program. The program calculates estimates of apparent Km and Vmax by linear regression using Lineweaver-Burke, Woolf and Hofstee plots. The choice of values given was made ensuring that residual errors were minimized and were randomly distributed. Significant differences were tested using the Student's t-test.

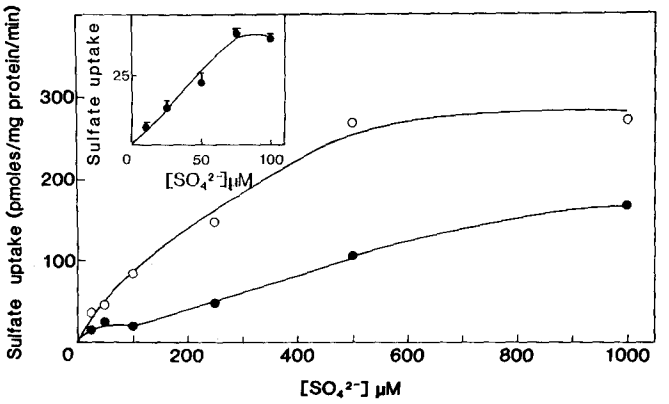

Fig. 1. The effect of the extracellular sulfate concentration on the initial rate of sulfate uptake in normal human skin fibroblasts. For uptake, the cells were incubated for up to 1 min in 1 ml medium containing various concentrations of [^{35}S]-Na_2SO_4, 150 mM NaCl, 1mM $MgCl_2$ and either 10 mM Tris Hepes, pH 7.5 (●) or 25 mM MES, 4.6 mM Tris, pH 5.5 (○); insert, experiment in the low range of SO_4^{2-} concentrations, carried out in a separate batch of cells.

RESULTS

Preliminary studies in skin fibroblasts have shown that sulfate uptake increases linearly with time over a period of up to 2 min. Therefore, initial rates of uptake were measured for no longer than 2 min. A typical experiment in the physiological range of extracellular sulfate concentrations, showing the effect of the extracellular SO_4^{2-} concentrations on the initial rate of sulfate uptake at pH 7.5 and 5.5 is given in Fig. 1. As we have previously observed in human lung fibroblasts (18), at pH 7.5 the uptake of sulfate in human skin fibroblasts displays two saturable components (Fig. 1, Table 1): a low capacity high-affinity component operating under conditions of sulfate deprivation ($[SO_4^{2-}]_{out}$ < 100 µM) and a high capacity low-affinity component in the physiological range of extracellular sulfate concentrations (SO_4^{2-} levels in human serum = 300 µM; (3)). As can be seen in the insert (Fig. 1), which presents results of a separate experiment in the low range of extracellular SO_4^{2-} concentrations, the high-affinity low-capacity system is consistently observed in the skin fibroblasts.

The effect of extracellular chloride and bicarbonate on sulfate uptake is given in Figs. 2A, 2B and results show that both anions inhibit sulfate uptake in a dose dependent manner. Kinetic studies at varying extracellular Cl^- concentrations ($[Cl^-]$=0,50,150 mM, gluconate replacing Cl^- at low Cl^- concentrations, 10 mM Tris Hepes pH 7.5) demonstrate an increase in the apparent Km of sulfate uptake with increased Cl^- levels: 0.5 ± 0.01, 1.3 ± 0.2, 2.9 ± 0.08 mM, respectively. Vmax is not significantly affected indicating that chloride inhibits sulfate uptake in a competitive manner. In

Table 1. The apparent K_m and V_{max} values for the sulfate transport system in skin fibroblasts isolated from normal and cystic fibrosis patients. Experiments were carried out as described in Fig. 1. Significant differences (*) were tested by student's t-test ($p < 0.001$). Results are expressed as the mean ± standard deviation (σ_{n-1}) of values obtained in several experiments. The total number of experiments and the number of age/sex matched pairs of cell lines in each case are given in that order in parenthesis under each result.

	Apparent K_m (μM)		V_{max} (pmoles/mg protein/min)	
	Normal	Cystic Fibrosis	Normal	Cystic Fibrosis
A. pH 7.5 (high affinity)	43.6 ± 23.0 (7,4)	40.0 ± 19.4 (7,4)	24.6 ± 7.1 (7,4)	27.9 ± 9.1 (7,4)
pH 7.5 (low affinity)	1740.0 ± 410.0 (4,2)	1800.0 ± 690.0 (4,2)	338.1 ± 36.3* (4,2)	675.0 ± 130.7* (4,2)
B. pH 5.5	237.8 ± 110.0 (5,4)	286.7 ± 130.5 (5,4)	275.1 ± 104.8* (5,4)	485.4 ± 45.6* (5,4)

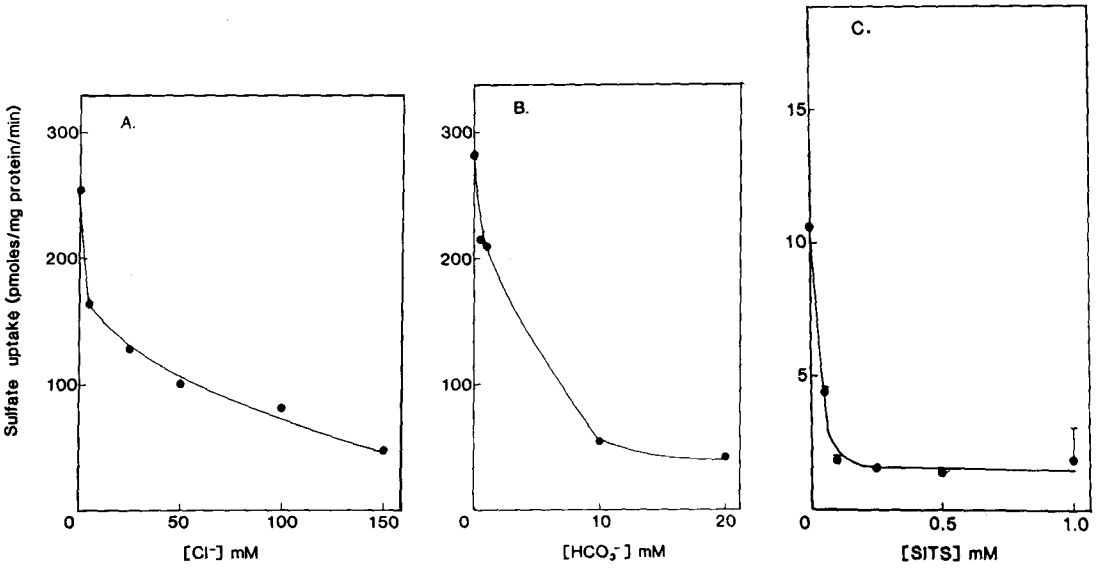

Fig. 2. Chloride, bicarbonate and the anion exchange inhibitor SITS inhibit sulfate uptake in normal human skin fibroblasts in a dose-dependent manner. A. The cells were incubated for 1 min in 1 ml medium containing 4 mM hemimagnesium gluconate, 10 mM Tris Hepes pH 7.5, 100 μM [^{35}S]-Na_2SO_4 and various ratios NaCl/Na gluconate up to a total of 150 mM. B. The cells were incubated for 1 min in 1 ml medium containing 4 mM hemimagnesium gluconate, 10 mM Tris Hepes pH 7.5, 100 μM [^{35}S]-Na_2SO_4 and various ratios $NaHCO_3$/Na gluconate up to a total of 150 mM. C. For SITS inhibition experiments, cells were first preincubated for 20 min at 37°C in a medium containing a salt mixture and glucose levels identical to those in the growth medium (1.8 mM $CaCl_2$, 0.25 mM $Fe(NO_3)_3 \cdot 9\ H_2O$, 5.4 mM KCl, 0.8 mM $MgCl_2$, 110 mM NaCl, 1 mM NaH_2PO_4, 25 mM D-glucose, 26 mM $NaHCO_3$, pH 7.5), and various concentrations of SITS. Sulfate uptake was then measured for 1 min in the same medium and 25 μM [^{35}S]-Na_2SO_4.

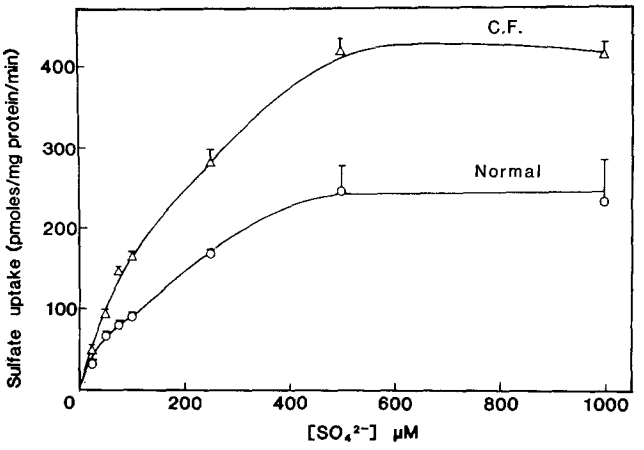

Fig. 3. The effect of the extracellular sulfate concentration on sulfate uptake at pH 5.5 in skin fibroblasts isolated from normal (○) and cystic fibrosis (△) patients. For uptake, the cells were incubated for up to 2 min in 1 ml medium containing various concentrations of [^{35}S]-Na_2SO_4, 150 mM NaCl, 1 mM $MgCl_2$, 25 mM MES, 4.6 mM Tris pH 5.5.

8 separate experiments, 50 mM extracellular chloride was consistently found to significantly stimulate sulfate efflux, albeit no more than 10-20%. SITS, a potent inhibitor of the anion exchange activity in erythrocytes (16) inhibits sulfate uptake into skin fibroblasts in a dose-dependent manner (Fig. 2C). Taken together, these data suggest that sulfate uptake in skin fibroblasts occurs via a SO_4^{2-}/Cl^- exchange mechanism.

Results in Fig. 1 suggest that low extracellular pH has a stimulatory effect on sulfate influx into skin fibroblasts. Further kinetic studies showed that lowering the extracellular pH from 7.5 to 5.5 caused a significant decrease in the apparent Km of sulfate influx (Table 1). These results suggested that pH is an important regulatory factor of sulfate transport and therefore comparisons between normal and CF skin fibroblasts were carried out both at pH 7.5 and pH 5.5.

Sulfate uptake at varying extracellular sulfate concentrations, at pH 7.5 and pH 5.5 was measured in skin fibroblasts isolated from normal and CF subjects. The most dramatic differences were observed at low extracellular pH and typical results are given in Fig. 3. Kinetic studies were carried out in several normal and CF skin fibroblast cell lines. Average values for apparent Km and Vmax obtained in these experiments are given in Table 1. In spite of the large errors associated with some of the constants and which indicate the degree of variability between the separate batches of cells used for these experiments, several conclusions can be drawn from these data. No significant difference can be observed at pH 7.5 between the kinetic constants of the high-affinity component of sulfate uptake in normal and CF skin fibroblasts. The Vmax of the low-affinity component of sulfate uptake is markedly stimulated in the CF cells. Moreover, at pH 5.5 when sulfate uptake appears

to occur mainly via the low affinity transport system (Fig. 1), the transport is stimulated in CF skin fibroblasts over the entire range of SO_4^{2-} concentrations tested (Fig. 3, Table 1). In conclusion, the V_{max} value for the high-capacity low-affinity sulfate transport system is significantly higher in CF cells, at both pH 7.5 and pH 5.5 (Table 1). The apparent K_m value is similar in normal and CF cells.

No significant difference could be demonstrated between sulfate efflux in skin fibroblasts isolated from normal and cystic fibrosis patients (results not shown). Since, as shown above, the capacity of sulfate influx is increased in CF, our results indicate a possible net increase in the intracellular availability of inorganic sulfate in CF.

DISCUSSION

Sulfate uptake in normal human skin fibroblasts was compared to uptake in skin fibroblasts isolated from cystic fibrosis (CF) patients. The high-affinity low-capacity sulfate uptake system which operates under conditions of sulfate deprivation does not appear to be affected in CF (Table 1). As indicated by higher V_{max} values (Table 1), the capacity of the low affinity system is significantly increased in CF, both at pH 7.5 and at pH 5.5. However, the affinity of this carrier system for sulfate does not appear to be affected in CF.

It may not be coincidental that the permeability to another anion, chloride, has also been found to be altered in CF. Thus chloride permeability via a chloride channel is reduced in CF respiratory epithelia (20), in the CF sweat duct (19) and in primary cultures of CF tracheal cells (21,22). Studies in trachea cells indicate that the regulation of chloride channel activity is defective in CF (22). It is conceivable that the chloride channel and the SO_4^{2-}/Cl^- exchange system may be regulated by a common control mechanism and that this regulatory mechanism is altered in CF. It is of interest to speculate that as a result of this altered regulatory system in CF, a modification of the anion exchanger may transform it from a carrier highly selective to Cl^- to a carrier more selective to SO_4^{2-}. Such a modification would increase the effective number of SO_4^{2-} exchangers at the expense of Cl^- exchangers, and would be expected to result in an increase in the capacity (V_{max}) of the sulfate transport system, as we observe in CF. An analogous transformation of an efficient Cl^- carrier to an efficient SO_4^{2-} carrier by chemical modification has been reported in red blood cells to explain the enhancement by several orders of magnitude of SO_4^{2-} uptake occurring concomitantly with Cl^- uptake inhibition, following dansylation of the red blood cell membrane (25).

In conclusion, we report an increase in the capacity of the sulfate uptake system in CF as compared to normal skin fibroblasts. Since sulfate efflux does not appear to be affected in CF and influx is stimulated, a net increase in sulfate uptake would be expected to occur in these cells, possibly causing an increase in the pool of intracellular sulfate available for sulfation processes. If present, a similar increased sulfate uptake in the CF tracheal epithelium, may explain the fact that tracheobronchial secretions of patients with CF are more highly sulfated and exhibit stronger acidic properties than those from normal patients (6,7,26) and that a significant correlation has been demonstrated between enhanced levels of highly sulfated mucins in tracheobronchial secretions of CF patients and increased severity of this disease (10).

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