ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 226, No. 2, October 15, pp. 657-665, 1983

Inhibition of Sugar Uptake by Ascorbic Acid in *Escherichia coli*

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Received April 19, 1983, and in revised form June 24, 1983

The uptake of glucose by the glucose phosphotransferase system in *Escherichia coli* was inhibited greater than 90% by ascorbate. The uptake of the nonmetabolizable analog of glucose, methyl-α-glucoside, was also inhibited to the same extent, confirming that it was the transport process that was sensitive to ascorbate. Similarly, it was the transport function of mannose phosphotransferase for which mannose and nonmetabolizable 2-deoxyglucose were substrates that was partially inhibited by ascorbate. Other phosphotransferase systems, including those for the uptake of sorbitol, fructose and *N*-acetylglucosamine, but not mannitol, were also inhibited to varying degrees by ascorbate. The inhibitory effect on the phosphotransferase systems was reversible, required the active oxidation of ascorbate, was sensitive to the presence of free-radical scavengers, and was insensitive to uncouplers. Because ascorbate was not taken up by *E. coli*, it was concluded that the active inhibitory species was the ascorbate free radical and that it was interacting reversibly with a membrane component, possibly the different enzyme IIB components of the phosphotransferase systems. Ascorbate also inhibited other transport systems causing a slight reduction in the passive diffusion of glycerol, a 50% inhibition of the shock-sensitive uptake of maltose, and a complete inhibition of the proton-symport uptake of lactose. Radical scavengers had little or no effect on the inhibition of these systems.

The chemistry and biochemistry of ascorbic acid have been widely studied, and it has gained notoriety in the controversy over its effects on the health of the human body. However, very little work has focused on the effect of ascorbate on bacteria, a common resident in the human gut. One of the few observations reported was that the addition of ascorbate to an aerobic culture of *Escherichia coli* caused an eightfold induction of catalase synthesis (1). This response was attributed to the production of hydrogen peroxide, an inducer of catalase synthesis (1-3), during the oxidation of ascorbate. A second result of ascorbate being added to the growth medium was a pause in growth regardless of the medium, followed by slower growth rates in some but not all media (1, 4). An investigation of the origin of this effect has progressed in a number of directions.

The experiments to be described in this paper consider the possibility that ascorbate was affecting sugar uptake and having an indirect effect on growth. Ascorbate is frequently used as an electron source, usually in the presence of phenazine methosulfate, to energize the uptake of sugars and amino acids into membrane vesicles (5, 6). Therefore, sugar-transport systems dependent upon a membrane potential such as the lactose proton-symport and melibiose ion-symport systems might be affected by ascorbate. In addition there is a small body of evidence suggesting that a membrane potential in some way inhibits glucose uptake by the phosphoenolpyruvate:sugar phosphotransferase system (7-9) which is the sole route for glucose uptake

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0003-9861/83 $3.00

in *E. coli* (10). Indeed, an inhibitory effect of ascorbate on methyl-α-glucoside uptake into membrane vesicles has been cryptically reported (11). This paper describes the effect of ascorbate on sugar uptake by *E. coli* and reveals that the transport systems of several sugars, including those for glucose and lactose, are inhibited by ascorbate.

MATERIALS AND METHODS

Bacterial strains. The following strains were used in this study: ZSC17 (*glk rps*L), ZSC103 (*glk, rps*L, *pts*G), and ZSC114 (*glk, rps*L, *pts*M) (12).

Media and growth conditions. Cells were grown at 37°C in M9 medium (Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1 g per liter H_2O; (13)) supplemented after autoclaving with 1 mM $MgSO_4$, 5 μg/ml thiamine hydrochloride, 0.2% w/v carbon source, 0.2% w/v casamino acids, and 25 μl trace-element solution ($FeSO_4 \cdot 7H_2O$, 2.5 g; H_3BO_3, 2.9 g; $CoSO_4 \cdot 7H_2O$, 1.2 g; $CuSO_4 \cdot 5H_2O$, 0.1 g; $MnCl_2 \cdot 4H_2O$, 0.09 g; H_2SO_4, 5.0 ml; $Na_2MoO_4 \cdot 2H_2O$, 2.5 g; $ZnSO_4 \cdot 7H_2O$, 2.1 g in 1 liter H_2O).

Preparation of cells for uptake studies. Cells were prepared for uptake experiments essentially as described by Postma (14). When cells reached midexponential phase they were collected by centrifugation, washed one time with M9 medium without supplements, and stored at 0°C until used, usually a period of not more than 2 h, in M9 medium supplemented with $MgSO_4$ and thiamine as described above. The cell suspension was aerated at 37°C for 6 min and the ^{14}C-labeled compound was added at zero time. Samples of 50 μl were removed at the times indicated, immediately filtered through Whatman nitrocellulose filters (0.45-μm pore size), and washed with 5 ml of 0.5 M LiCl. The filters were dissolved in 5 ml of Brays scintillant (naphthalene, 240 g; PPO (2,5-diphenyloxazole), 16 g; dimethyl-POPOP (2,2'-*p*-phenylene-bis (4-methyl-5-phenzyloxazole)), 0.8 g; ethylene glycol, 80 ml; methanol, 400 ml; and 1,4-dioxane up to 4 liters) and counted in a scintillation counter. Background radioactivity on filters was determined in a similar manner in the absence of cells. The rate of uptake is expressed as nanomoles of ^{14}C-labeled substrate taken up per minute per milligram dry cell weight at 37°C. It should be noted that because of the transient nature of the inhibitory effect, particularly at low ascorbate concentrations, the uptake experiments were started within 15 s of the addition of ascorbate and only the 30-s and 1-min time points were used for rate calculations.

Efflux experiments. Cells were prepared as described for the uptake experiments and loaded with ^{14}C-labeled sugar in a 6-min incubation at 37°C. The cells were collected by centrifugation for 3 min in an Eppendorf centrifuge at top speed. Time zero for efflux was the time when the cells were resuspended by vortexing in new M9 medium, supplemented with $MgSO_4$ and thiamine. Samples of 50 μl were removed and filtered as already described.

Chemicals. L-[*carboxyl*-^{14}C]ascorbic acid (17 mCi/mmol), 2-deoxy-D-[U-^{14}C]glucose (318 mCi/mmol), D-[U-^{14}C]fructose (283 mCi/mmol), *N*-acetyl-D-[U-^{14}C]glucosamine (57.9 mCi/mmol), D-[U-^{14}C]glucose (336 mCi/mmol), [U-^{14}C]glycerol (171 mCi/mmol), D-glucose-[1-^{14}C]lactose (59 mCi/mmol), D-[U-^{14}C]mannose (270 mCi/mmol), D-[U-^{14}C]mannitol (59 mCi/mmol), [U-^{14}C]maltose (604 mCi/mmol), methyl(α-D-[U-^{14}C]gluco)pyranoside (279 mCi/mmol), and D-[U-^{14}C]sorbitol (333 mCi/mmol) were obtained from Amersham. *N,N,N',N'*-tetramethylethylenediamine (TEMED)[2] was obtained from Baker. All other biochemicals were obtained from Sigma.

RESULTS

Effect of ascorbate on the uptake of glucose and non-metabolizable glucose analogs. The addition of 1 mM ascorbate to whole cell suspensions inhibited the apparent uptake of ^{14}C-labeled glucose by greater than 90% (Fig. 1a). Because ascorbate could have also affected the catabolism of glucose and thereby changed its apparent uptake and accumulation, the effect of ascorbate on the uptake of the nonmetabolizable analogs of glucose, methyl-α-glucoside and 2-deoxyglucose, was studied revealing a strong inhibition of the former (Fig. 1b) but very little effect on the latter (Fig. 1c). The rate of uptake of 2-deoxyglucose was slowed slightly but the final amount of label accumulated was unchanged whereas both the rate of uptake and level of accumulation of methyl-α-glucoside were reduced by greater than 90%. Because methyl-α-glucoside is transported only by the glucose phosphotransferase and 2-deoxyglucose is transported only by the mannose phosphotransferase system, the former system involving enzyme IIB^{Glc} and enzyme III^{Glc} was much more sensitive to ascorbate than the latter.

[2] Abbreviations: DNP, dinitrophenol; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; TEMED, *N,N,N',N'*-tetramethylethylenediamine; DTT, dithiothreitol.

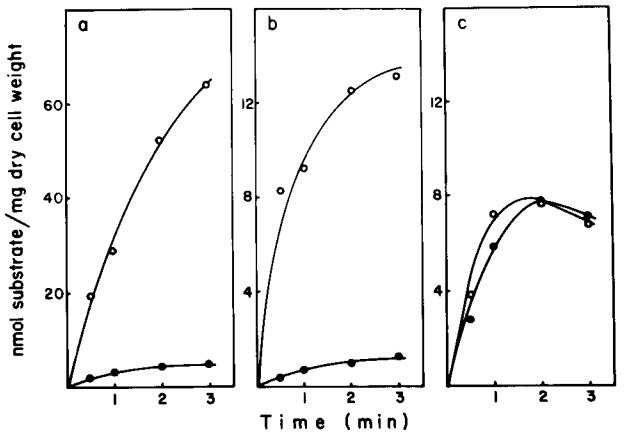

FIG. 1. Effect of 1 mM ascorbate on the uptake of ^{14}C-labeled glucose (a), methyl-α-glucoside (b), and 2-deoxyglucose (c) by *E. coli* ZSC17 grown in M9 medium with 0.2% glucose as carbon source. The labeled sugar, 46 μM, was added at 0 time without ascorbate (○) or with ascorbate added 15 s earlier (●).

To confirm this observation, the effect of ascorbate on glucose uptake in *pts*G (ZSC103; enzyme IIB^{Glc} deficient) and *pts*M (ZSC114; enzyme IIB^{Man} deficient) strains was studied (Fig. 2). In the *pts*M mutant where glucose transport was entirely dependent upon the glucose phosphotransferase system, the extent of ascorbate inhibition was greater than in the wild-type strain. In the *pts*G mutant where the mannose phosphotransferase system was responsible for glucose uptake, ascorbate inhibited the initial rate of glucose uptake to the same extent as the initial rate of 2-deoxyglucose uptake in the wild-type strain (Fig. 1c). Only the final levels of accumulation differed, perhaps the result of the metabolism of the glucose or differences in efflux.

To test the explanation that ascorbate was affecting the rates of uptake by stimulating the rate of efflux, cells were loaded with labeled sugar and the efflux of label was followed with and without ascorbate (Fig. 3). Ascorbate had very little effect on the efflux of labeled methyl-α-glucoside or 2-deoxyglucose (data not shown) but did have a small stimulatory effect on the efflux of glucose or its catabolism products, although such small differences could not explain the 80–90% inhibition of sugar uptake via the glucose phosphotransferase system.

The dissimilarity in molecular structures between glucose and ascorbate suggested that the inhibition was not the result of competitive binding of ascorbate to the transport enzymes and, upon investigation, the initial rates of uptake of both glucose and methyl-α-glucoside were noncompetitively inhibited with no change in the apparent K_m values of 14 μM for glucose or 180 μM for methyl-α-glucoside.

Requirement for ascorbate oxidation in the inhibition of glucose phosphotransferase. The extent of the inhibition of sugar uptake was dependent upon the amount of ascorbate present up to concentrations of 1 mM where maximal inhibition was observed. Despite this relationship between the amount of ascorbate and the extent of inhibition, molecular ascorbate was not the inhibitory species because the prevention of ascorbate oxidation by the inclusion of dithiothreitol or the chelating reagent EDTA prevented the inhibition of sugar uptake (Table I). The fact that dithiothreitol, a strong reducing agent, did not affect sugar uptake (Table I) suggested that more than a simple reduction or oxidation was involved in the ascorbate effect. Furthermore, hydrogen peroxide, a product of as-

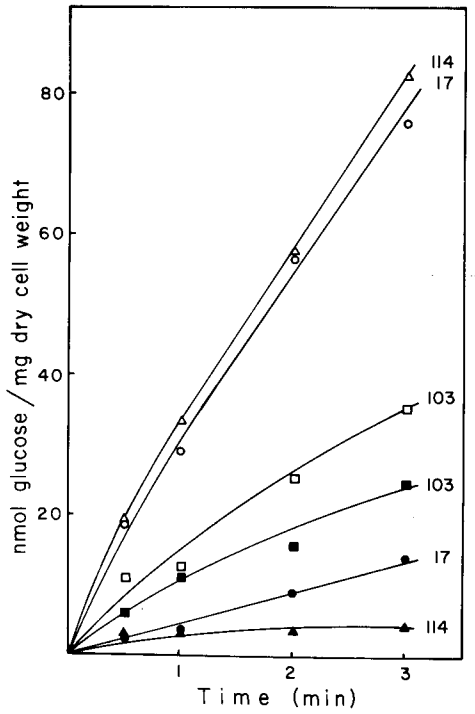

FIG. 2. Effect of 1 mM ascorbate on the uptake of ^{14}C-labeled glucose by *E. coli* strains ZSC17 (parental; ○), ZSC114 (*pts*M; △) and ZSC103 (*pts*G; □). The labeled glucose, 46 μM, was added at 0 time without ascorbate (open symbols) or with ascorbate added 15 s earlier (closed symbols). The strains ZSC17 and ZSC114 were grown in M9 medium with 0.2% glucose as carbon source while strain ZSC103 was grown in M9 medium with 0.2% mannose as carbon source.

corbate oxidation, was not the inhibitory species because 1 mM H_2O_2 had no effect on sugar uptake even in the presence of 0.1 mM $FeSO_4$ (Table I).

If the cell suspension was incubated for several minutes with ascorbate prior to the addition of labeled sugar, the inhibitory effect of ascorbate was reduced. Clearly, active oxidation of the ascorbate was required for the inhibition to occur, and when the oxidation process was complete, the reversible nature of the effect allowed sugar uptake to return to normal. This suggested that the inhibitory species was an intermediate formed in the oxidation of ascorbate, possibly the ascorbate free radical. Its involvement was further suggested by the effect on the ascorbate inhibition by the amines Tris and TEMED, which can act as free-radical carriers and scavengers (15). They did not affect sugar uptake when added alone and when added with ascorbate they overcame the inhibitory effect of ascorbate on both glucose and methyl-α-glucoside uptake (Table II). Unlike EDTA and dithiothreitol, these amines did not prevent ascorbate oxidation but significantly enhanced the rate of ascorbate oxidation consistent with their role as free-radical scavengers or carriers (Table III). Despite this significant enhancement of the oxidation rate, less than 5% of the total ascorbate, starting at 1 mM, would have been oxidized before the labeled sugar was added and not more than 30% would have been oxidized in a 3-min incubation.

To determine whether the ascorbate free radical was acting as an external agent on a membrane component such as enzyme

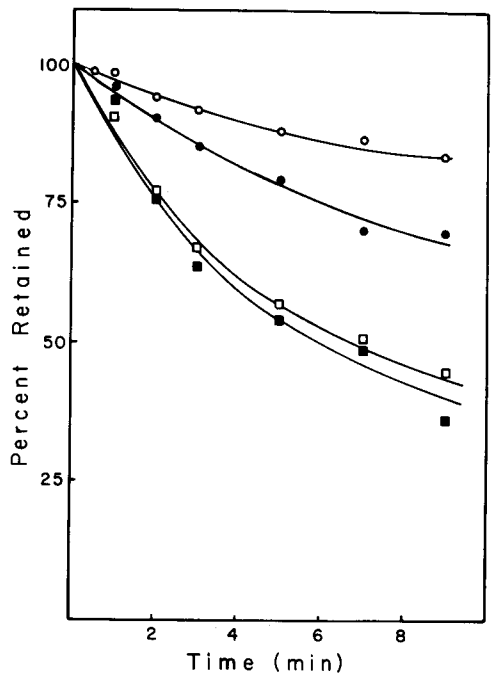

FIG. 3. Effect of 1 mM ascorbate on the efflux of ^{14}C label from cultures of ZSC17 loaded with [^{14}C]glucose (○) or [^{14}C]methyl-α-glucoside (□). Time 0 was the point at which the loaded cells were resuspended by vortexing in M9 medium supplemented with thiamine and $MgSO_4$ without ascorbate (open symbols) or with ascorbate (closed symbols).

TABLE I

EFFECT OF VARIOUS REAGENTS ON GLUCOSE UPTAKE AND ASCORBATE INHIBITION OF GLUCOSE UPTAKE BY *E. coli*

Reagent	Ascorbate (μM)	Glucose uptake (nmol/min/mg dry cell weight)
—	—	43.80
—	1	12.16
—	25	4.28
EDTA (0.1 mM)	25	44.94
DTT (1 mM)	—	45.09
DTT (1 mM)	25	43.90
H_2O_2 (1 mM)	—	41.30
H_2O_2 (1 mM) + $FeSO_4$ (0.1 mM)	—	41.75
Ammonium persulfate (1 mM)	—	44.51

IIB^{Glc} or internally on enzyme III^{Glc}, the uptake of labeled ascorbate was studied directly (Table IV). No accumulation of label was evident over a range of ascorbate concentrations from 0.26 to 1.1 mM although the same batch of cells was very active in transporting glucose.

Involvement of membrane potential in ascorbate inhibition of the glucose phosphotransferase system. Membrane potential has been implicated as a factor capable of inhibiting methyl-α-glucoside uptake (7, 8, 9). Furthermore, ascorbate has been used in conjunction with phenazinemethosulfate to energize sugar and amino acid uptake in membrane vesicles (5, 6), suggesting a possible mechanism for ascorbate inhibition of glucose uptake. If a membrane potential were being created by ascorbate, uncouplers such as dinitrophenol (DNP) and carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) should prevent the ascorbate effect. Consistent with the observations of other workers (10), the uncouplers alone inhibited apparent glucose uptake, did not affect 2-deoxyglucose uptake, and stimulated methyl-α-glucoside uptake (Fig. 4). The latter effect was shown to be the result, at least in part, of a much slower efflux of methyl-α-glucoside in the presence of an uncoupler (Table V). When ascorbate was included in the uptake experiments with CCCP, its inhibitory effect on both glucose and methyl-α-glucoside uptake was enhanced and there was a reduction in the amount of 2-deoxyglucose accumulated (Fig. 4). Only the data for CCCP are shown but the data for DNP are qualitatively and quantitatively the same. With membrane energization eliminated as a possible mechanism for the ascorbate effect, we have concluded that there is a direct interaction of the ascorbate radical with a component of the glucose phosphotransferase system or a membrane component closely associated with the system.

TABLE II

EFFECT OF THE AMINES TRIS AND TEMED ON THE ASCORBATE INHIBITION OF SUGAR UPTAKE IN *E. coli*

Ascorbate (mM)	Amine, pH 7.0 (0.1 M)	Nmol/mg dry cell weight: Glucose uptake	Nmol/mg dry cell weight: Methyl-α-glucoside uptake
—	—	39.57	9.30
1.0	—	3.32	0.56
1.0	TEMED	30.02	11.73
1.0	Tris	34.38	11.00

TABLE III

EFFECT OF VARIOUS REAGENTS ON THE OXIDATION OF 1 mM ASCORBATE IN M9 MEDIUM pH 7.0 AS DETERMINED USING A CLARKE ELECTRODE[a]

Reagent	Rate of ascorbate oxidation (nmol/min/ml)
—	13.3
EDTA (0.1 mM)	3.4
DTT (1 mM)	6.4
TEMED (0.1 M, pH 7.0)	105.3
Tris (0.1 M, pH 7.0)	34.3
TEMED (0.1 M, pH 7.0) + DTT (1 mM)	8.6

[a] The rate of ascorbate oxidation in a 1 mM ascorbate solution was determined by following the rate of oxygen disappearance in a 2-ml reaction volume in a Gilson oxygraph equipped with a Clarke electrode. A stoichiometric relationship between ascorbate oxidation and oxygen disappearance was used.

TABLE IV

UPTAKE OF ^{14}C-LABELED ASCORBATE AND ^{14}C-LABELED GLUCOSE BY *E. coli*

Experiment	Labeled sugar	Concentration	Time of incubation (min)	^{14}C label accumulated (nmol)
1	Glucose	46 μM	0.5	20.27
			3.0	94.39
2	Ascorbate	0.26 mM	0.5	0.0
			3.0	0.0
3	Ascorbate	1.12 mM	0.5	0.0
			3.0	0.0

Effect of ascorbate on the uptake of other sugars. The effect of ascorbate on the apparent uptake of sugars transported by other phosphoenolpyruvate:sugar phosphotransferase systems is shown in Fig. 5. A range of inhibition from nil in the case of mannitol to greater than 90% in the case of sorbitol was found. The rate of mannose uptake was inhibited about the same amount as the rates of 2-deoxyglucose uptake and glucose uptake in the *pts*G mutant. As discussed above, the difference in accumulation between mannose and 2-deoxyglucose was probably the result of sugar catabolism in the former case. In all cases where ascorbate inhibited sugar uptake, the amines Tris or TEMED either fully or, as in the case of sorbitol, partially prevented the inhibition suggesting that the mechanism of inhibition was similar for all of the phosphotransferase systems.

To test the universality of the ascorbate effect, its influence on the uptake of sugars by other transport systems was investigated. Glycerol uptake involves a facilitated-diffusion mechanism which was only slightly inhibited by ascorbate (Fig. 6a). Maltose uptake is by a shock-sensitive sys-

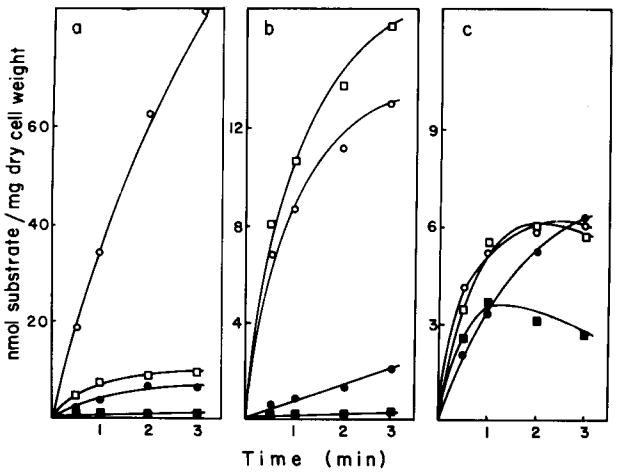

FIG. 4. Effect of the uncoupler CCCP on ascorbate inhibition of the uptake of ^{14}C-labeled glucose (a), methyl-α-glucoside (b), and 2-deoxyglucose (c) by *E. coli* ZSC17 grown in M9 medium with 0.2% glucose as carbon source. The labeled sugar, 46 μM, was added at 0 time with no ascorbate or CCCP (○), with 1 mM ascorbate added 15 s earlier (●), with 20 μM CCCP added 30 s earlier (□), or with 20 μM CCCP and 1 mM ascorbate added 30- and 15 s earlier, respectively (■). When CCCP was replaced with 1 mM DNP the results were essentially the same.

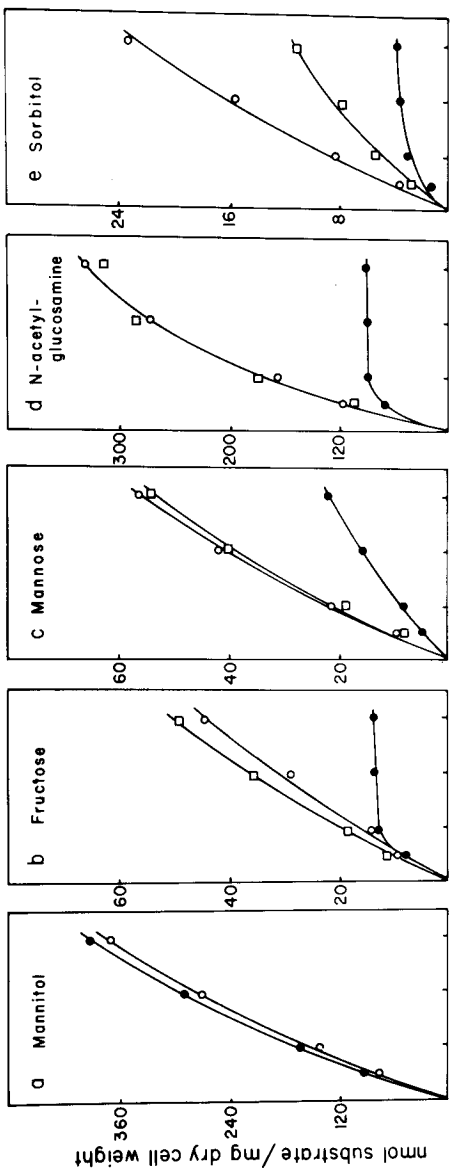

FIG. 5. Effect of 1 mM ascorbate on the uptake of ^{14}C-labeled mannitol (a), fructose (b), mannose (c), *N*-acetylglucosamine (d), and sorbitol (e) by *E. coli* ZSC17 in M9 medium, pH 7.0 (○), and in M9 medium containing 0.1 M TEMED, pH 7.0 (□). The labeled sugar, 46 μM, was added at 0 time without (○) or with ascorbate added 15 s earlier (● or □). The uptake of labeled sugar was unaffected by TEMED in the absence of ascorbate, and 0.1 M Tris, pH 7.0, had the same effect as TEMED. The cells in each section were grown in the same sugar as that used in the uptake experiments.

TABLE V

EFFECT OF DNP ON THE EFFLUX OF METHYL-α-GLUCOSIDE FROM *E. coli*

Experiment	DNP concentration (mM)	Ascorbate concentration (mM)	Percentage of ^{14}C-label remaining after 9 min
1	—	—	42
2	—	1	36
3	1	—	84
4	1	1	30

tem powered by chemical energy and it was also partially inhibited by ascorbate (Fig. 6b). The most pronounced ascorbate effect was on lactose uptake which involves a proton-symport system driven by a protonmotive force (Fig. 6c). Unlike their effect on the ascorbate inhibition of the phosphotransferase systems, Tris and TEMED had no effect on ascorbate inhibition of glycerol or lactose uptake and only a partial effect on the inhibition of maltose uptake.

DISCUSSION

Ascorbate inhibited the apparent uptake of sugars by the various phosphotransferase systems with the extent of inhibition ranging from greater than 90% in the case of glucose phosphotransferase to nil in the case of the mannitol phosphotransferase. In the two systems where nonmetabolizable substrates were available, methyl-α-glucoside for the glucose phosphotransferase and 2-deoxyglucose for the mannose phosphotransferase, the uptake of both metabolizable and nonmetabolizable substrates were similarly affected by ascorbate consistent with transport being the primary process affected by ascorbate. Unfortunately, nonmetabolizable substrates were not available for all of the phosphotransferase systems and this prevented an unambiguous statement as to the target of ascorbate in those systems. However, because the amine radical scavengers antagonized the ascorbate effect on all of the phosphotransferase sugars, including glucose and mannose where nonmetabolizable analogs had confirmed transport to be the target process for ascorbate, it can be argued that the principal mode of ascorbate action on the other phosphotransferase systems was also on the transport process. The observed variations in inhibition were the result of varying sensitivities of the different systems to ascorbate.

The inhibitory effect of ascorbate was

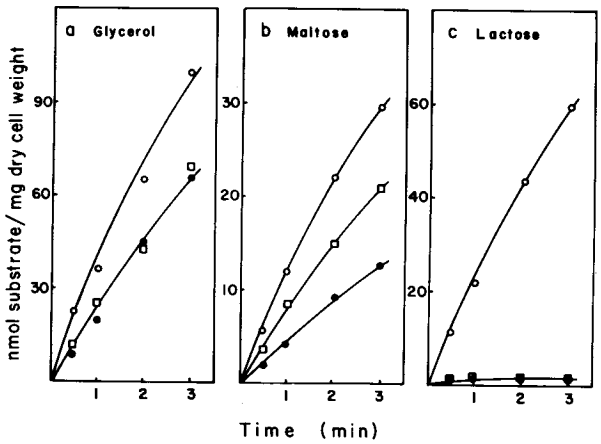

FIG. 6. Effect of 1 mM ascorbate on the uptake of ^{14}C-labeled glycerol (a), maltose (b), and lactose (c) in M9 medium, pH 7.0 (○), and M9 medium with 0.1 M TEMED, pH 7.0 (□). The labeled sugar, 46 μM, was added at 0 time without ascorbate (○) or with ascorbate added 15 s earlier (● or □). The uptake of labeled sugar was unaffected by TEMED in the absence of ascorbate, and 0.1 M Tris, pH 7.0, had the same effect as TEMED. The cells in each section were grown in the same sugar as that used in the uptake experiments.

not restricted to the phosphotransferase system. The facilitated-diffusion uptake of glycerol, the shock-sensitive uptake of maltose, and the proton symport of lactose were all sensitive to ascorbate to varying degrees. By focusing on the effect of radical scavengers, it is clear that the mechanism of ascorbate inhibition is different in each case, consistent with the different mechanisms of uptake. Further study of the reason for the extreme sensitivity of lactose uptake to ascorbate may ultimately shed light on the mechanism of proton symport.

From the observations that the inhibition of the phosphotransferase systems was reversible, required an active oxidation process, was sensitive to free-radical scavengers, and that ascorbate was not taken up by the cells, a mechanism involving an ascorbate radical interaction with a membrane component, perhaps enzyme IIB, is proposed. The differences in the extent of inhibition could, therefore, be explained by the differing affinities of the various enzyme IIB proteins for the ascorbate radical. Alternatively, the ascorbate radical could have been interacting with some other unidentified membrane component which exerted varying degrees of influence on the different enzyme IIB proteins. The reversibility of the inhibition indicated that the radical did not covalently bind to its target, but whether the noncompetitive mechanism involved electron donation by the free radical or a simple binding was not clear. However, if electron donation to a membrane component were taking place, it did not result in membrane energization because uncouplers did not antagonize the inhibitory effect.

Several workers have recently noted that uncouplers stimulate the uptake of methyl-α-glucoside by *E. coli* (10, 11), and this has been attributed to a decrease in the apparent K_m of the phosphotransferase system for methyl-α-glucoside from 200 to 20 μM in the presence of uncouplers (10). The decreased rate of efflux of methyl-α-glucoside in the presence of uncouplers observed in this paper suggested an alternative or perhaps complementary explanation previously demonstrated by earlier work (16, 17).

ACKNOWLEDGMENTS

This work was supported by Grant A9600 and a Postgraduate Scholarship to H.E.R. from the Natural Sciences and Engineering Research Council of Canada. We thank J. Switala for technical assistance.

REFERENCES

1. RICHTER, H. E., AND LOEWEN, P. C. (1981) *Biochem. Biophys. Res. Commun.* **100,** 1039–1046.
2. HASSAN, H. M., AND FRIDOVICH, I. (1978) *J. Biol. Chem.* **253,** 6445–6450.
3. YASHPE-PURER, Y., HENIS, Y., AND JASHPE, J. (1977) *Canad. J. Microbiol.* **23,** 84–91.
4. RICHTER, H. E., AND LOEWEN, P. C. (1982) *Arch. Biochem. Biophys.* **215,** 72–77.
5. KONINGS, W. H., BARNES, E. M., JR., AND KABACK, H. R. (1971) *J. Biol. Chem.* **246,** 5857–5861.
6. KONINGS, W. N., AND FREESE, E. (1972) *J. Biol. Chem.* **247,** 2408–2418.
7. DILLS, S. S., APPERSON, A., SCHMIDT, M. R., AND SAIER, M. H., JR. (1980) *Microbiol. Rev.* **44,** 385–418.
8. HERNANDEZ-ASENSIO, M., RAMIREZ, J. M., AND DEL CAMPO, F. F. (1975) *Arch. Microbiol.* **103,** 155–162.
9. DEL CAMPO, F. F., HERNANDEZ-ASENSIO, M., AND RAMIREZ, J. M. (1975) *Biochem. Biophys. Res. Commun.* **63,** 1099–1105.
10. POSTMA, P. W., NEYSSEL, O. M., AND VAN REE, R. (1982) *Eur. J. Biochem.* **123,** 113–119.
11. REIDER, E., WAGNER, E. F., AND SCHWEIGER, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5529–5533.
12. CURTIS, S. J., AND EPSTEIN, W. (1975) *J. Bacteriol.* **122,** 1189–1199.
13. MILLER, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
14. POSTMA, P. W. (1977) *J. Bacteriol.* **129,** 630–639.
15. PRYOR, W. A. (1966) Free Radicals, McGraw-Hill, New York.
16. HOFFEE, P., ENGLESBERG, E., AND LANG, F. (1964) *Biochim. Biophys. Acta* **79,** 337–350.
17. HALPERN, Y. S. AND LUPO, M. (1966) *Biochim. Biophys. Acta* **126,** 163–167.