determined using a Knauer osmometer (Table III). The results show that imipramine has surfactant properties. Equipment for precise determinations of critical micelle concentration was not available but it would appear to lie between 50 and 80 m*M*.

The turbidity of various solutions containing bilirubin and desipramine was measured at 650 nm (a minimum in the bilirubin absorption spectrum). Between 10 and 50 m*M* desipramine the solution was grossly turbid but above 80 m*M* the solution was clear, probably because bilirubin was taken up into desipramine micelles (Table IV).

Discussion. The results show that both imipramine and desipramine exhibit a strong activating effect on UDP glucuronyl transferase. The effect occurs over a narrow range of drug concentrations and was not due to a direct chemical effect of the drugs on bilirubin since it was not observed in control experiments carried out in the absence of microsomal suspension.

Imipramine and desipramine are surfactants and the effects may be explained on the basis of surfactant-induced absorption. In vitro, imipramine molecules will concentrate at interfaces between the microsomes and the surrounding liquid medium. Polar groups will be orientated towards the aqueous medium and the non-polar parts of the molecules directed towards the lipid protein matrix of the microsomes. This may accelerate the absorption of bilirubin by the microsomes. When the surfaces become saturated with imipramine molecules the molecules must enter the main bulk of the solution and at this stage bilirubin is precipitated.

At drug concentrations exceeding the critical micelle concentration bilirubin is taken up into the imipramine micelles. Unfortunately, it was not possible to investigate this latter condition in the full reaction medium so the effect on the production of bilirubin conjugates is not known.

Marked activation of glucuronyl transferase and other microsomal enzymes by surfactants has been reported[8–12]. Since the completion of this work MULDER[13] has also reported activation of UDP glucuronyl transferase by desipramine. Our work has shown that imipramine and desipramine act as cationic surfactants in aqueous media. It is possible that the drugs produce changes in the microsomal environment of the enzyme and hence make it more accessible for the substrate.

The activation effect was more pronounced when bilirubin was solubilized in a serum-EDTA medium. This may indicate that other factors are concerned in the reaction. It is possible that the drugs are bound to serum proteins, notably albumin, in preference to bilirubin and this may cause more rapid transfer of the bilirubin to microsomal protein.

The effects measures in this in vitro system may not be of significances in vivo, particularly since high drug concentrations were used. However, imipramine and desipramine can produce a conjugated hyperbilirubinaemia often accompanied by cell damage, and jaundice may disappear without cessation of therapy. It is possible that imipramine and desipramine may cause a transient over production of conjugated bilirubin by the factors described above.

Table III. Osmolality of imipramine solutions

Imipramine concentration (m*M*)	Osmolality (m.osmol./kg)
1	2
2	4
5	10
10	20
25	50
50	78
100	85
150	95
200	100
250	100
300	100

Table IV. Effect of desipramine on the turbidity of a bilirubin solution

Desipramine concentration (m*M*)	E_{560}
0	0.065
1	0.540
10	0.650
25	0.650
50	0.650
75	0.650
100	0.174
150	0.125
200	0.140

Résumé. Nous avons recherché l'action de l'imipramine et de la désipramine sur l'UDP glucuronyle transférase dans les microsomes hépatiques. Les concentrations de ces drogues entre 1 et 5 m*M* ont activé l'enzyme. Ce sont des surfactants cationiques.

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Glucose Uptake by *Aspergillus nidulans*, Purification and Properties of Glucose Binding Protein

Inspite of significant advance in elucidation of uptake systems in bacteria[1–3] and yeast[4,5], relatively little is known concerning sugar transport in fungi. An impressive body of evidence has accumulated which indicates that sugar transport in bacteria[6] and yeast[7,8] is coupled with phosphorylation; however, this is not true in case of filamentous fungi *A. nidulans*[9] and *Neurospora crassa*[10].

Studies conducted in our laboratory indicated the relationship between biotin status of *A. nidulans* and the cellular permeability to ammonium ions[11,12]. In an earlier

Table I. Purification of glucose binding protein from *A. nidulans*

Fraction	Total units[a]	Protein (mg)	Sp. activity (units[a]/mg protein)	Purification (folds)
Purification from normal cells				
Crude	44.4	39.0	1.139	–
0.5–0.7 $(NH_4)_2SO_4$ saturation	39.7	4.8	8.271	7.266
Sephadex G (75–40) 9th fraction	38.2	0.6	63.660	55.930
Purification from biotin deficient cells				
Crude	40.4	42.00	0.962	–
0.5–0.7 $(NH_4)_2SO_4$ saturation	35.6	4.90	7.263	7.550
Sephadex G (75–40) 9th fraction	34.0	0.58	58.620	60.940

[a] Unit can be defined as the amount of protein required to bind 1 μmole of D-glucose/24 h at 4 °C.

publication[13] we showed that biotin deficiency in *A. nidulans* resulted in to decrease uptake of glucose. Further, the uptake system for glucose transport was characterized as a constitutive, against concentration gradient and energy dependent.

As a result of more recent studies, it is now generally accepted that uptake of many substances by bacteria requires specific carrier or binding protein[14,15], they are located in the 'periplasmic region' of the cell[16,17] and can be selectively released by cold osmotic shock treatment[15]. Only few reports have appeared which indicated the involvement of binding protein in fungal transport systems[18,19]. Earlier, we presented evidence for involvement of binding protein in glucose uptake by *A. nidulans*[13]. The present communication deals with the purification and properties of the binding protein and its role in glucose transport during biotin deficiency in *A. nidulans*.

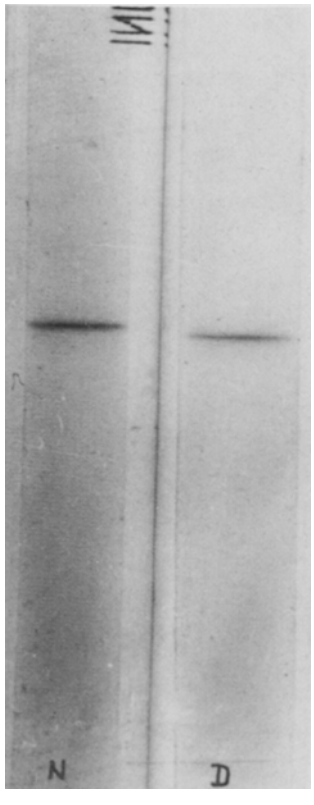

Fig. 1. Gel electrophoretic pattern of glucose-binding protein isolated from normal (N) and biotin deficient (D) *A. nidulans*.

The strain, composition of basal media and cultural conditions used in the present investigation were same as described earlier[13]. D-isomers of sugars were used in this study.

Isolation and assay of glucose-binding protein were performed by two-stage cold osmotic shock treatment, according to the procedure of WILEY[18], and equilibrium dialysis technique of BARASH and HALPERN[20] respectively, with slight modification as described in our earlier publication[13]. Binding protein was purified by ammonium sulfate precipitation and gel filtration techniques. Precipitates obtained after 0.5–0.7 $(NH_4)_2SO_4$ saturation, were dissolved in *tris*-HCl buffer pH 7.0 and loaded on sephadex G75–40 column (1 × 40 cm). Gel filtration was facilitated with the help of 0.01 *M* potassium phosphate buffer (pH 7.0) containing 1 m*M* 2-mercaptoethanol. Fractions of 5 ml each were collected at a flow rate of 1 ml per 10 min.

In general, the method of DAVIS[21] was followed for polyacrylamide gel electrophoresis of binding protein. Gels containing 7.5% (w/v) acrylamide and 10% (w/v) bisacrylamide were polymerized chemically with potassium persulfate in an anaerobic condition. Spacer and sample gels were omitted. Protein (100 μg) was loaded on the top of the gel and electrophoresis was carried out in

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0.05 *M tris*-glycine buffer (pH 8.4) at 4 °C. Total current of 1.5 mA/gel for initial 15 min was applied and finally increased to 4 mA/gel. Bromophenol blue was used as a tracer dye. Gels were stained with coomassie blue and destained by repeated washings in 7.5% (v/v) acetic acid.

Earlier we have presented evidence for involvement of binding protein in the transport of D-glucose in *A. nidulans*. Using ammonium sulfate precipitation and gel filtration techniques, glucose-binding protein has been purified to about 60-fold (Table I). When purified protein was subjected to gel electrophoresis, it showed a single band (Figure 1). The molecular weight of binding protein isolated from normal and biotin deficient cultures was identical, about 29,200. Most of the purified binding proteins have a molecular weight between 28,000 and 42,000; one exception may be the tryptophan-binding protein isolated by WILEY[18] from *N. crassa* which has the molecular weight of 200,000.

Table II. Effect of PCMB, 2, 4 DNP, KCN and NaN_3 on glucose binding activity of binding protein

Additions (1 m*M*)	Inhibition (%)	
	Normal	Deficient
PCMB	95.0	92.0
2,4 DNP	5.2	5.0
KCN	5.6	6.1
NaN_3	6.1	6.0

Table III. Effect of various sugars on the activity of glucose binding protein

Additions	Sp. activity (U/mg protein)	
	Normal	Deficient
Control	63.6	58.6
Fructose	63.0	58.4
Galactose	53.0	50.0
Mannose	55.6	51.3
Arabinose	62.8	58.2
Sucrose	63.0	58.1
Lactose	63.5	58.0

Sugars added as 40 µmoles/assay system.

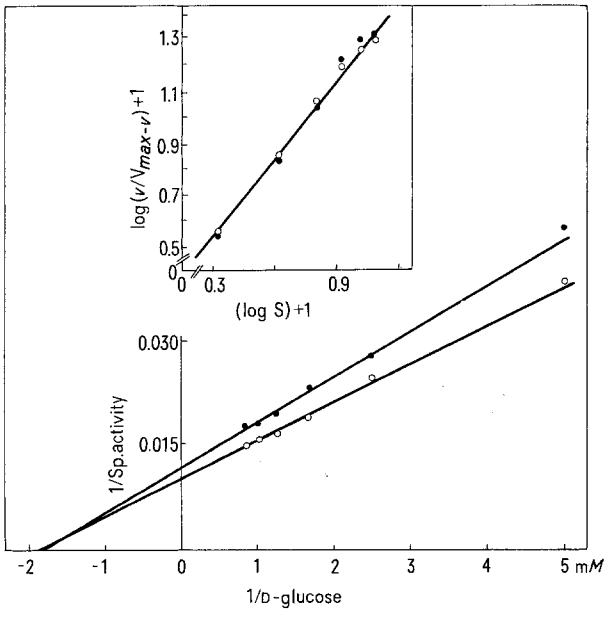

Fig. 2. Lineweaver Burk plot of glucose-binding protein of normal (⊙) and biotin deficient (●) *A. nidulans*. Inset figure is the Hill plot.

The result in Table II shows that the binding of glucose to binding protein required-SH group, and is an energy-independent process. SH group-containing carrier proteins needed for transport of aminoacids and sugars have been reported[22]. Requirement of energy source for transport and not for binding is a well-established fact. All sugars tested, except galactose and mannose, did not inhibit the activity of glucose-binding protein (Table III). The results are in line with those reported[13,23–25]. Binding protein lost about 77% activity when assayed at 35 °C. The pH optima for glucose-binding protein was found to be 6.75.

Lineweaver Burke plot of glucose-binding protein showed an idential affinity constant of 5.26×10^{-4} *M* for binding proteins isolated from normal and deficient *A. nidulans* (Figure 2). This suggested that the decrease in the ability to takeup glucose by biotin deficient cells appeared not to be correlated with the affinity of glucose-binding protein to glucose. The results are in agreement with those observed in in vivo[13], except that the *Km* value observed here is slightly lower. The possibilities for the observed difference in the *Km* values between transport and binding protein have been very well discussed by BARASH and HALPERN[20]. When these data were replotted as a Hill plot (inset Figure 2), they showed slope value of one in either case, indicating a single site for glucose on binding protein.

The binding proteins isolated from normal and biotin deficient *A. nidulans* have been found to possess similar properties, except that the activity of binding protein isolated from biotin deficient cells is slightly lower than the binding protein preparation from the normal culture. Addition of biotin in the medium resulted in increased uptake of glucose by deficient cells. However in vitro experiment did not show any change in the activity of the binding protein by avidin or biotin (unpublished data). Thus the effect of biotin on glucose uptake may be of indirect nature.

Zusammenfassung. Nachweis, dass gereinigtes, D-Glukose bindendes Protein aus der Biotin-Mangelmutante von *Aspergillus nidulans* an der Gel-Elektrophorese einen einzigen Ring zeigte. Das Protein ist hitzeunbeständig und hat einen *Km*-Wert von $5{,}26 \times 10^{-4}$ *M* und seine Aktivität wurde, mit Ausnahme von D-Galaktose und D-Mannose, durch verschiedene Zucker nicht beeinträchtigt.

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