J. Enzyme Inhibition, 1993, Vol. 6, pp. 271–282
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EXPLORING THE HEXOKINASE GLUCOSE BINDING SITE THROUGH CORRELATION ANALYSIS AND MOLECULAR MODELING OF GLUCOSAMINE INHIBITORS

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(*Received 2 June 1992*)

A series of N-substituted glucosamines has been designed, synthesized, and tested as inhibitors of yeast hexokinase. All derivatives exhibited competitive inhibition kinetics with respect to glucose. Quantitative structure-activity relationships were derived from the resulting inhibition data. The most significant equation demonstrated the existence of highly specific steric effects for the seven *meta*-substituted benzoylglucosamines included in the relationship. Molecular modeling of potential complexes between the inhibitors and the hexokinase substrate binding site strongly suggests that the steric effects arise from potential contacts with two amino acid residues lying in the region occupied by the amide substituents.

KEY WORDS: Yeast hexokinase, N-acylglucosamines, quantitative structure-activity relationships, molecular modeling.

INTRODUCTION

Hexokinase (ATP: D-hexose 6-phosphotransferase, EC 1.7.1.1) catalyzes the ATP dependent phosphorylation of hexoses with D-glucose serving as the most significant substrate. The reaction facilitates the accumulation of glucose within the cell and provides glucose-6-phosphate required for glycolysis, glycogenesis, and the pentose-phosphate pathway. Thus hexokinase is particularly crucial to normal as well as neoplastic tissues which depend upon high rates of glycolysis to satisfy energy requirements and therefore may be a potential target for cancer chemotherapy. Inhibition of hexokinase could disrupt the fuel supply and therefore the cell growth of tumors. However a beneficial effect can only be realized if an inhibitor is selective for neoplastic cell hexokinase. To develop such selectivity, potential binding interactions in the enzyme active site and adjoining regions must be systematically surveyed and characterized. Furthermore, tumor cell hexokinase must be compared with other hexokinases to delineate advantageous differences. This can best be accomplished through the study of structure-activity relationships for well designed series of inhibitors. To lay the proper groundwork for the future study of tumor cell hexokinases, it was desirable to initiate investigations with a series of congeneric inhibitors using the well characterized yeast hexokinase as a model system. Since the

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X-ray structure for this enzyme is known, structure-activity relationships should be amenable to detailed interpretation and therefore should provide a sound basis for comparison with hexokinases of unknown structure. An early study by Maley and Lardy[1] established that *N*-acyl-2-amino-2-deoxyglucose analogs, *N*-acylglucosamines, were competitive inhibitors of yeast hexokinase as well as bovine brain hexokinase. Subsequent X-ray crystal studies[2,3] have shown that glucose analogs bind in the same orientation as glucose in the substrate-hexokinase complex. Thus, an expansion of the *N*-acylglucosamine series may allow analysis of the physicochemical binding characteristics of defined regions in or near the yeast hexokinase substrate binding site. The design, synthesis, and evaluation of an extended set of inhibitors is the subject of this report.

MATERIALS AND METHODS

Materials

Yeast hexokinase Type V (Lot. 96F-8110; Cat. No. H-5250), glucose-6-phosphate dehydrogenase Type XV (Lot. 128F-8125; Cat. No. G-6378), ATP, and $NADP^+$ were purchased from Sigma Chemical Corp. *N*-Acetyl-D-glucose, D-glucosamine hydrochloride, and all other synthetic reagents were obtained from Aldrich Chemical Co. *p*-Nitrophenol was purified by recrystallization from toluene before use.

Hexokinase Inhibition

The hexokinase reaction was followed by coupling with glucose-6-phosphate dehydrogenase[4] and measuring the rate of NADPH formation at 340 nm with an LKB Ultrospec K 4053 spectrophotometer. The assay solution consisted of 0.1 M triethanolamine hydrochloride (pH 8.0), 5 mM $MgCl_2$, 0.4 mM $NADP^+$, 3.7 mM ATP, 0.5 unit/ml glucose-6-phosphate dehydrogenase, and 0.03 unit/ml yeast hexokinase. Temperature was maintained at 25°C. Six glucose concentrations, in duplicate, ranging from 0.075 to 2.4 mM were used for each kinetic determination run in the presence of three to four inhibitor concentrations. Under these conditions the K_m for glucose was found to be 0.1383 ± 0.0094 mM. All rate data were analyzed using the microcomputer program of Lutz, Bull, and Rodband.[5]

p-*Nitrophenyl-phenylacetate*

A solution of *p*-nitrophenol (12.0 g; 0.088 M), phenylacetic acid (10.0 g; 0.0734 M), and 4-dimethylaminopyridine (0.2 g; 0.0016 M) in 200 ml ethyl acetate was chilled in an ice bath with magnetic stirring. Dicyclohexylcarbodiimide (18.0 g; 0.087 M) was added in portions. The resulting mixture was allowed to warm to room temperature and stirred for 24 h with exclusion of moisture. The precipitated dicyclohexylurea was removed by filtration with washing by ethyl acetate. The combined ethyl acetate solutions were washed with 4 × 100 ml portions of 5% Na_2CO_3 and 4 × 100 ml portions of saturated NaCl before drying over anhydrous Na_2SO_4. Filtration and evaporation, *in vacuo*, afforded 17.87 g (94%) crude *p*-nitrophenyl-phenylacetate which solidified on standing. The product could be used without further purification but was normally recrystallized once from isopropanol; mp 61–62°C (lit.[6] 62–63°C). IR: 1763 cm^{-1} (ester carbonyl).

N-*phenylacetyl-D-glucosamine* (***9***)

Triethylamine (0.50 g; 0.0049 M) was added dropwise to a magnetically stirred solution of D-glucosamine hydrochloride (1.07 g; 0.0049 M) and *p*-nitrophenyl-phenylacetate (1.93 g; 0.0075 M) in 25 ml dimethylsulfoxide at room temperature. The resulting yellow solution was stirred for 24 h then added dropwise with stirring to 200 ml methylene chloride. The cloudy mixture was allowed to stir for 1 h, then filtered to isolate the precipitated crude product. The initially waxy solid was washed extensively with methylene chloride until a light white powder was obtained. Drying overnight in a desiccator afforded 1.0 g (68%) crude *N*-phenylacetyl-D-glucosamine, which moved as a single spot on tlc (silica gel; 20% MeOH–$CHCL_3$). Purification was achieved by recrystallization from absolute ethanol; mp 215–217°C (Lit.[1] 208–210°C). ^{1}H NMR (360 MHz, D_2O): 7.35 (m, 5H, Phenyl), 5.19 (d, 0.67H, $J_{1,2}$ = 3.5 Hz, 1-H α-anomer), 4.69 (d, 0.33H, $J_{1,2}$ = 8.4 Hz, 1-H β-anomer), 3.95 (dd, 1H, $J_{2,3}$ = 9.8 Hz, 2-H), 3.90 (ddd, 1H, $J_{5,6a}$ = 3.8, $J_{5,6b}$ = 2.4 Hz, 5-H), 3.83 (dd, 1H, $J_{3,4}$ = 9.0 Hz, 3-H), 3.83 (m, 2H, CH_2-Phenyl), 3.68 (m, 2H, 6a, 6b-H), 3.49 (dd, 1H, $J_{4,5}$ = 9.9 Hz, 4-H). IR: 1647, 1624 cm^{-1} (amide carbonyl). Found: C, 56.59; H, 6.51; N, 4.76. $C_{14}H_{19}NO_6$ requires C, 56.55; H, 6.40; N, 4.71%.

Molecular Modeling

All ten inhibitor structures were subjected to molecular mechanics minimization using MMX87, a microcomputer version of MM2.[7,8] Each minimized structure was then submitted to a conformational search via CAMSEQPC[9,10] in which the amide bond was fixed in the preferred *trans* conformation while the bonds on either side were allowed to rotate through 360° in 10° increments as illustrated in Figure 1. In the acetyl derivative, **10**, only one rotatable bond was of interest while in the phenylacetyl, **9**, one additional bond, $-CH_2-C_6H_5$, was included in the analysis. Furthermore, the phenoxy linkages of **3**, were also examined.

The coordinates for yeast hexokinase[2,3] were obtained from the Protein Data Bank[11–13] and read into the SYBYL molecular modeling package[14] running on a Silicon Graphics Personal IRIS workstation.

Statistical Analysis

Equations between structural properties and enzyme inhibition were developed by standard regression analysis. The entry of variables into the regression equations was controlled manually to allow close examination of the effects of each of the molecules and each of the physicochemical variables under study.

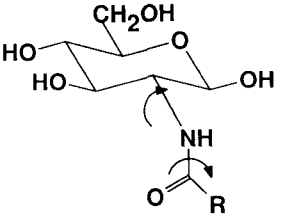

FIGURE 1 Bonds allowed to rotate in CAMSEQ conformational search.

RESULTS

Design

Inhibitor structures were selected to provide the largest feasible range in physicochemical properties in an effort to ensure that changes in inhibitor potency could be identified with specific changes in structural features. Thus lipophilicity as characterized by the Hansch π constant varies from -0.12 (3-CN, **5**) to 2.39 (3-OC_6H_5, **3**) while electronic properties range from 0.71 (3-NO_2, **2**) to -0.07 (3-CH_3, **7**) in terms of Hammett σ_m. Steric bulk in terms of molecular polarizability or molecular volume varies extensively as well. Variation in substitution at the *meta* position of the aromatic ring of *N*-benzoyl-glucosamines was chosen specifically because the possibility of ring rotation would allow exploration of a larger area adjacent to the hexokinase glucose binding site.

Synthesis

A number of methods for the N-acylation of glucosamines have been reported. Reaction with acid chlorides or acid anhydrides would provide the desired analogs. However these reactions are best carried out on penta-*O*-acetyl glucosamine, thus necessitating several additional steps of protection and deprotection. Reaction between appropriate *p*-nitrophenyl esters and unprotected D-glucosamine offered the most efficient means of generating the required series of *N*-acylglucosamine congeners.[15] The *p*-nitrophenyl esters were prepared by reaction between *p*-nitrophenol and the appropriate carboxylic acid in the presence of dicyclohexyl-carbodiimide with 4-dimethylaminopyridine as catalyst. Treatment of D-glucosamine hydrochloride in dimethylsulfoxide with the *p*-nitrophenyl ester in the presence of triethylamine (Figure 2) then afforded the N-acylglucosamines in 40–90% crude yields (Table I). 4-Dimethylaminopyridine was examined as an alternative base to neutralize the glucosamine hydrochloride however this led to considerable difficulties in product purification. The reaction was conducted at room temperature and could be followed by disappearance of glucosamine as indicated by a negative ninhydrin test on silica gel thin layer chromatography. In most cases, the reaction was complete after 8 to 10 hours but was routinely allowed to proceed for 24 hours.

Enzyme Inhibition

Coupling of hexokinase to glucose-6-phosphate dehydrogenase allowed the reaction to be monitored by following the formation of NADPH at 340 nm in the ultraviolet. Inhibitors were dissolved in dimethylsulfoxide which had no effect on hexokinase at cuvette concentrations less than 10%. The inhibitors were examined for effects on

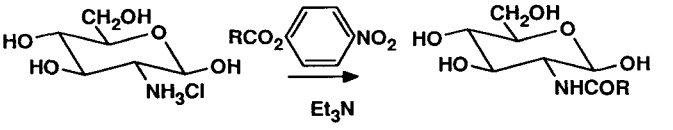

FIGURE 2 Reaction of glucosamine with *p*-nitrophenylesters.

TABLE I
Chemical and analytical data *N*-R-glucosamines

No.	R	% Yield	Recryst. solvent	MP °C (lit)	Anal.[a]
1	C_6H_5CO	75	95% EtOH	198–200 (198–200)[b]	—
2	3-NO_2-C_6H_4CO	43	95% EtOH	215–216 (215–216)[c]	—
3	3-OC_6H_5-C_6H_4CO	46	95% EtOH	222–224	C, H, N
4	3-F-C_6H_4CO	69	95% EtOH	220–221	C, H, N
5	3-CN-C_6H_4CO	37	MeOH	201–203	C, H, N
6	3-OCH_3-C_6H_4	81	MeOH	200–202	C, H, N
7	3-CH_3-C_6H_4CO	89	MeOH	200–202	C, H, N
8	3-Br-C_6H_4CO	88	EtOH	216–217 (221–222)[c]	C, H, N[d]
9	C_6H_5-CH_2CO	68	EtOH	215–217 (208–210)[c]	C, H, N[d]

[a]All elemental analyses were within 0.4% of theoretical. [b]Reference 15. [c]Reference 1. [d]Elemental analyses were determined because of the significant differences in melting points as compared to the literature.

the glucose-6-phosphate dehydrogenase reaction. No discernible effect on the dehydrogenase reaction was observed so that all inhibition could be attributed to action on the hexokinase alone. Inhibition kinetics were established by conducting the reaction in the presence of excess ATP while varying the amounts of glucose and of inhibitor. As reported by other laboratories,[1] the inhibition of hexokinase by N-acylglucosamines was found to be competitive with respect to glucose as illustrated by the Lineweaver-Burke plot for *N*-(3-nitrobenzoyl)-glucosamine (**2**) (Figure 3). The apparent inhibition constants K_i, were computed by a nonlinear regression procedure and are summarized for the ten derivatives in Table II. Seven of the eight benzoyl analogs were effective inhibitors of the enzyme with the electronegative 3-bromo, **8**, 3-cyano, **5**, and 3-nitro, **2**, affording the most potent inhibition. The 3-phenoxy derivative, **3**, exhibited only weak inhibition as did the acetyl, **10**, and phenacetyl, **9**, analogs. These results parallel those reported by Maley and Lardy[1] in the sense that **2** and **8** were the most active while **9** and **10** were the least active at single test concentrations. Unfortunately, K_i values were not determined by Maley and Lardy thus precluding direct comparison of results.

Quantitative Structure-Activity Relationships

Eight of the inhibitors in Table II are derivatives of *N*-benzoylglucosamine in which the only structural change occurs at the three position of the benzoyl ring. Thus it was possible to characterize the structural changes for these eight analogs in terms of physicochemical substituent constants. Regression analysis was then applied to study potential relationships between changes in hexokinase inhibition and changes in physicochemical properties. Four types of properties were examined. The Hansch

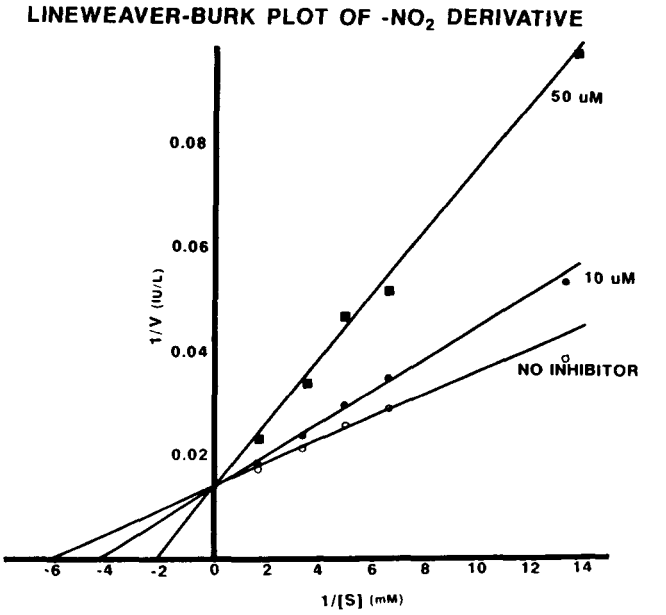

FIGURE 3 Lineweaver-Burke plot demonstrating competitive inhibition of yeast hexokinase by *N*-(3-nitrobenzoyl)glucosamine (**2**).

TABLE II
Physicochemical and biological data *N*-R-glucosamines

No.	R	K_i[a]	pK_i obs[b]	pK_i calc	MR[d]	B_1[e]
1	C_6H_5CO	0.333 (0.029)	3.48	3.52	0.103	1.00
2	3-NO_2-C_6H_4CO	0.045 (0.066)	4.34	4.38	0.736	1.70
3	3-OC_6H_5-C_6H_4CO	2.007 (0.213)	2.68[f]	3.95	2.768	1.35
4	3-F-C_6H_4CO	0.125 (0.013)	3.90	3.95	0.092	1.35
5	3-CN-C_6H_4CO	0.042 (0.002)	4.37	4.26	0.633	1.60
6	3-OCH_3-C_6H_4CO	0.107 (0.009)	3.97	3.95	0.787	1.35
7	3-CH_3-C_6H_4CO	0.060 (0.002)	4.22	4.16	0.565	1.52
8	3-Br-C_6H_4CO	0.024 (0.001)	4.62	4.69	0.888	1.95
9	C_6H_5-CH_2CO	3.221 (0.223)	2.49[f]	—	—	—
10	CH_3CO	1.605 (0.102)	2.79[f]	—	—	—

[a]Yeast hexokinase inhibition constant in mM (std. error). [b]Log $1/K_i$ where K_i is molar. [c]Log $1/K_i$ computed from equation (4). [d]Molar refractivity taken from reference 17 and scaled by 0.1. [e]Verloop value for minimum width of substituent in angstroms. Values taken from reference 17. [f]Not included in the derivation of equation (4).

π constant, calculated from CLOGP[16] for *meta*-substituted benzamides, characterized substituent lipophilicity. The Hammett σ constant[17] was employed as an indicator of electronic influences on the benzoyl ring. Field and resonance effects in the form of the Swain and Lupton electronic parameters[17] were also examined. Steric properties were represented by the Verloop STERIMOL parameters:[18] L for substituent length, B_1 for minimum substituent width, and B_4 for maximum substituent width. Finally molar refractivity, MR,[17] was considered as an indication of substituent polarizability although this parameter also parallels substituent bulk. Equations were derived between hexokinase inhibition and each parameter alone as well as all reasonable combinations of parameters. From this analysis it was evident that no statistically significant relationship existed between either lipophilic properties, π, or between electronic properties in the form of σ, field, or resonance effects and enzyme inhibition. However, changes in molar refractivity did reflect changes in hexokinase inhibition as indicated by equations (1) and (2).

$$\log 1/K_i = 0.45(0.52)MR + 4.32(0.66) \quad (1)$$

$$n = 8 \quad r = 0.613 \quad s = 0.529 \quad F = 3.61$$

$$\log 1/K_i = 1.54(1.11)MR - 0.67(0.36)MR^2 + 3.55(0.53) \quad (2)$$

$$n = 8 \quad r = 0.942 \quad s = 0.246 \quad F = 19.62$$

$$MR(opt) = 1.15(0.65 - 1.37)$$

In these equations enzyme inhibition is in the form $\log 1/K_i$ so that larger values represent higher activity. The numbers in parentheses are the 95% confidence intervals for the coefficients, n is the number of derivatives included in the equation, r is the correlation coefficient, s is the standard deviation, and F is the value used for an F test of significance. Although the linear relationship, equation (1), is not significant, it does provide an initial indication that MR may account for some of the changes in inhibition. Meanwhile, equation (2) is a parabolic relationship in MR which is highly significant and suggests that increases in molar refractivity do give increases in hexokinase inhibition until substituent MR reaches a value of 1.15, roughly equivalent to an ethyl group. While equation (2) appears to characterize the data very well, a plot of MR versus $\log 1/K_i$ showed that the 3-phenoxy analog, **3**, with low activity and high MR, was the sole reason for the parabolic correlation. Thus, additional derivatives with high MR values would be needed to substantiate this relationship. Removal of **3** from the data set and recomputation of the correlations reveals equations (3) and (4) as the most significant.

$$\log 1/K_i = 0.94(0.80)MR + 3.62(0.50) \quad (3)$$

$$n = 7 \quad r = 0.803 \quad s = 0.246 \quad F = 9.07$$

$$\log 1/K_i = 1.23(0.26)B_1 + 2.29(0.39)$$

$$n = 7 \quad r = 0.984 \quad s = 0.074 \quad F = 152.11 \quad (4)$$

Although the linear correlation with MR alone now provides a reasonable relationship, equation (4) with substituent minimum width, B_1, is clearly superior. The excellent correlation with B_1 strongly suggests that much of the apparent influence of MR is not due to polarizability but rather to fairly specific steric effects, at least for the substituents included in this analysis.

Molecular Modeling

The quantitative structure-activity relationships represented by equations (1)–(4) provide a physicochemical description of interactions at the hexokinase glucose binding site. Examination of potential fit between inhibitors and enzyme through the use of computer graphics afforded a means of visualizing the quantitative relationships. One of the available yeast hexokinase crystal structures includes a bound inhibitor, *o*-toluoylglucosamine, in the substrate binding site. This provides an indication of the inhibitor location and orientation within the hexokinase substrate binding site. While it was a trivial matter to superposition the glucose ring of the inhibitors onto that of the bound *o*-toluoylglucosamine to achieve a reasonable binding mode, it was evident that the conformation of the benzoyl groups might be critical in explaining the inhibition data. In order to assess the extent of conformational freedom available to the various benzoyl groups, all inhibitors were subjected to a conformational search using CAMSEQPC. In all cases this afforded two "families" of conformers defined by the orientation of the amide bond with respect to the glucose ring, one with the amide N–H pointing down (α) and one with the amide N–H pointing up (β). Within each family, the phenyl-carbonyl bond can be freely rotated with only very minor changes in conformational energy. Thus, it was evident that the hexokinase bound conformation of *o*-toluoylglucosamine, with the N–H of the *trans* amide bond pointing in the α direction represents one of the two sets of energetically feasible conformations and that corresponding conformations of the inhibitors under investigation could be used to examine binding interactions. The three bonds involved, glucose–NH–CO–R, were set to the torsion angles observed in the hexokinase-inhibitor crystal structure and these conformations subjected to molecular mechanics minimization using MMX87. Replacement of the hexokinase-bound *o*-toluoylglucosamine with the energy-minimized structures by glucose superpositioning allows the N-acyl portion of compounds **1**–**10** to fit into an open cleft adjacent to the glucose binding site. These enzyme-inhibitor complexes are exemplified by the relatively active 3-nitro- and 3-cyano-benzoylglucosamines (**2** and **5**) and by the relatively inactive 3-phenoxybenzoyl and phenacetyl derivatives (**3** and **9**) shown in Figures (4)–(7). These figures give a close-up of the hexokinase-inhibitor binding site with much of the enzyme structure removed to provide a clearer view of those aminoacid residues that could potentially come into direct contact with a bound inhibitor. Each figure is oriented looking along an axis defined by the R–CO–NH–glucose bonds with the benzoyl (or phenacetyl) substituent closest to the viewer and the glucose farthest away. Examination of the distances between binding site residues and various portions of the inhibitors suggested that hexokinase residues 153 (norvaline) on one side and 274 (lysine) on the other side of the amide substituents lie closest to the inhibitor phenyl rings and may be principally involved in the steric interactions identified in the regression analysis. Residues 153 and 274 as well as the inhibitors are shown with van der Waals dot surfaces to assist in visualizing the space occupied. Figures 4 and 5 illustrate the 3-nitro, **2**, and 3-cyano, **5**, analogs respectively, in the active site. Distance measurements show that there is more than enough space to accommodate the benzoyl ring and the substituents of these two highly active inhibitors. As shown in Figure 4, the nitro group projects toward the viewer with the oxygen atoms lying about 3.2 Å above lysine 274. Rotation of the phenyl ring could bring the electronegative nitro group even closer to the presumably positively charged lysine. In Figure 5, the linear cyano group is over 4 Å away from both residues 153 and

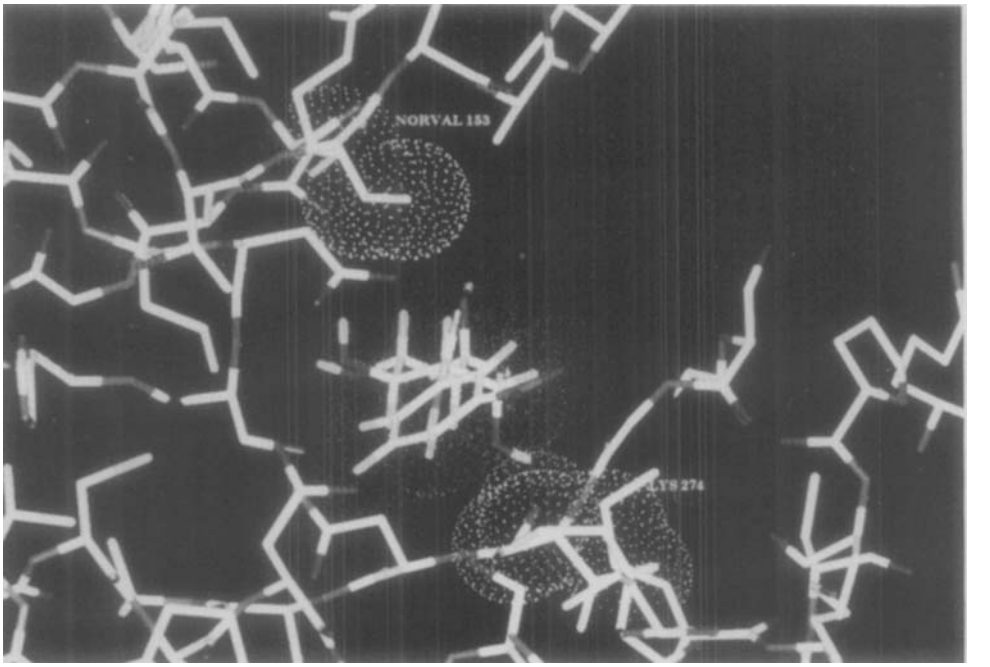

FIGURE 4 Potential complex of *N*-(3-nitrobenzoyl)glucosamine (**2**) in the yeast hexokinase substrate binding site. (See Colour Plate at rear)

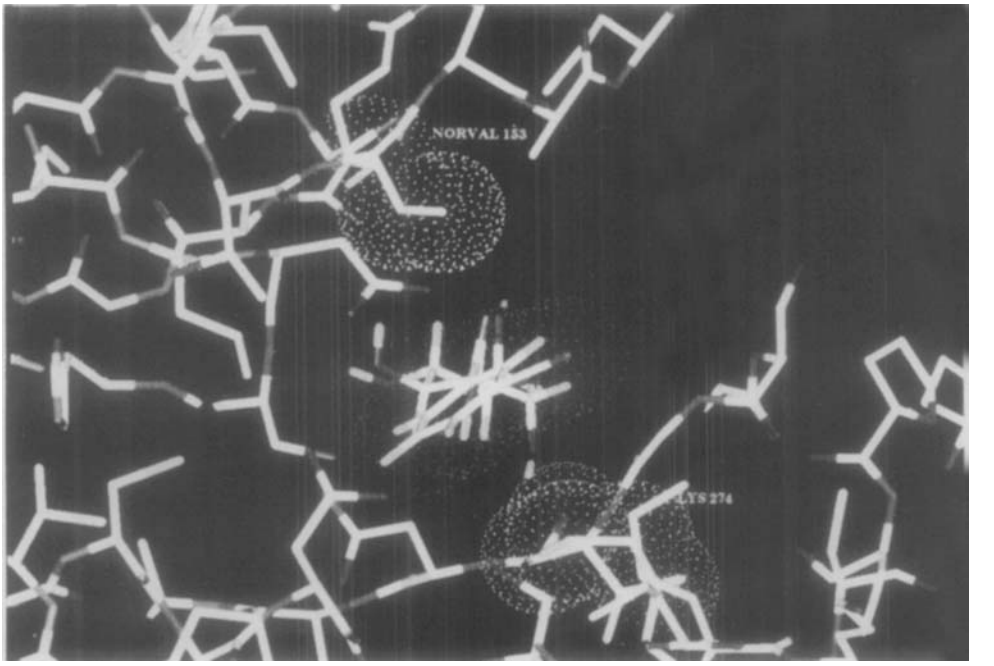

FIGURE 5 Potential complex of *N*-(3-cyanobenzoyl)glucosamine (**5**) in the yeast hexokinase substrate binding site. (See Colour Plate at rear)

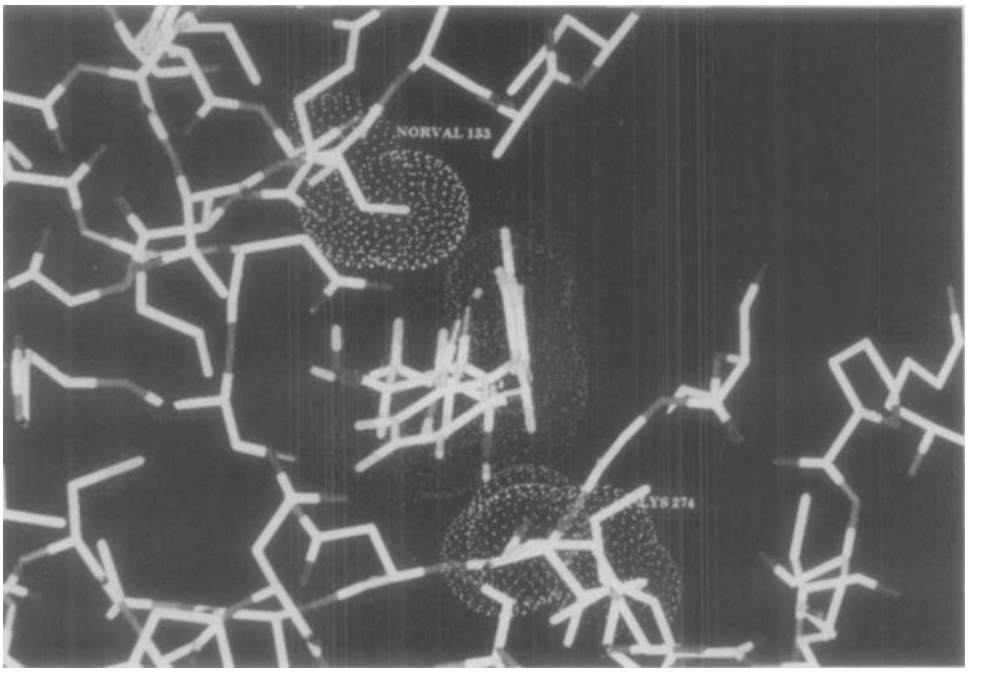

FIGURE 6 Potential complex of *N*-(3-phenoxybenzoyl)glucosamine (**3**) in the yeast hexokinase substrate binding site. (See Colour Plate at rear)

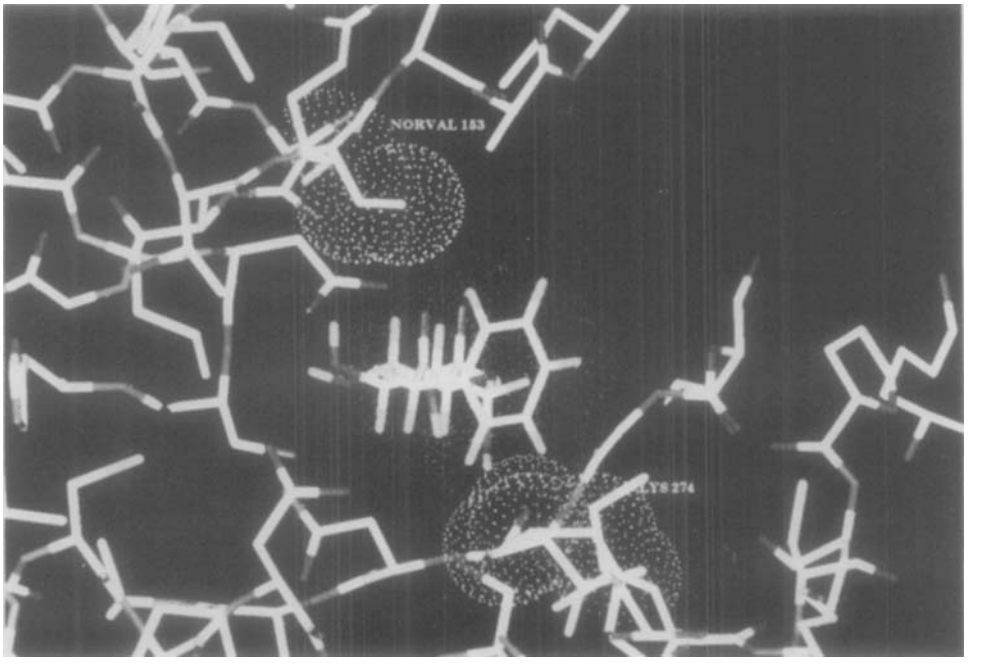

FIGURE 7 Potential complex of *N*-phenacetylglucosamine (**9**) in the yeast hexokinase substrate binding site. (See Colour Plate at rear)

274. The positive contribution of these substituents to inhibition may in part be due to a beneficial electrostatic attraction between lysine and the electronegative nitro or cyano although the overriding influence must be due to enzyme conformational changes elicited by these groups. Conversely, while the 2 less active derivatives can also fit into the binding site (Figures 6 and 7), the added bulk of the phenoxy and the flexible phenacetyl afford a much tighter fit. The phenoxy lies within 3 Å of the lysine and 2 Å of the norvaline while the phenacetyl phenyl ring is within 1.5–2.0 Å of the lysine. Binding of these derivatives may in fact require too great a conformational change in the enzyme without the benefit of added positive attractions within the active site.

DISCUSSION

Reported comparisons of X-ray crystal structures for native hexokinase with those for sugar complexes have indicated that the protein structure undergoes numerous changes upon substrate binding.[2] These changes were similar but not identical for substrates versus inhibitors thus explaining, at least in part, the observation that glucosamine analogs, while binding to the same site, do not serve as substrates. In the current study a series of acylated glucosamines have been designed, synthesized and tested as inhibitors with the objective of exploring the binding site and further elucidating physicochemical effects on binding to hexokinase. The development of quantitative structure-activity relationships for the inhibitors afforded the result that minimum width, B_1, for the various substituents best accounted for observed changes in inhibitory potency (equation (4)). While four of the seven inhibitors included in equation (4) incorporate symmetrical substituents with equivalent widths and lengths, the remaining three do not. Interpretation of the regression analysis results must then invoke specific, beneficial steric effects on enzyme conformation which involve orientation of the inhibitor *meta*-substituted benzoyl ring such that a minimum but yet significant change in protein structure is elicited. It seems that these steric effects, or more precisely contacts with hexokinase residues, must occur from above or below the plane defined by the benzoyl ring and not from extensions of substituent structure in the plane of the benzoyl ring since the lengths of substituents such as the linear cyano have no apparent effect on inhibition. Conformational changes in enzyme and inhibitor during inhibitor interaction are clearly limited since only those benzoylglucosamines with relatively small substituents gave effective enzyme inhibition. The *N*-acetyl analog, **10**, exhibited only weak inhibition, perhaps because the acyl group is too small to cause the required beneficial structural changes. The 3-phenoxybenzoyl congener, **3**, and the phenacetyl congener, **9**, were specifically designed to explore the enzyme surface for potential hydrophobic binding pockets. These obviously do not exist within the regions potentially occupied by these groups since these derivatives are only weak inhibitors. Since these two analogs are apparently not capable of eliciting beneficial conformational changes in the enzyme or are not capable of adopting a conformation which effectively binds to the enzyme, it is probable that the ability of the yeast hexokinase to accommodate larger substituents has been exceeded. These interpretations were largely supported by molecular modeling studies in which two of the better inhibitors were contrasted with two of the least active. It should be noted that the hexokinase coordinates employed were those obtained following binding with *o*-toluoylglucosamine and therefore do not reflect the specific conformational changes that may result from binding of the

inhibitors in the current study. Nevertheless it was obvious that the glucose binding pocket could easily allow interaction with energetically favorable conformations of the benzoylglucosamines with the notable exception of the 3-phenoxybenzoyl, **3**, and phenacetyl, **9**, derivatives where several potentially detrimental steric interactions were detected. Moreover, the modeling studies clearly suggested that electronegative substituents may experience some attraction for a lysine residue present in the region near the 3-position of the benzoyl ring. If such electrostatic forces do play a role in enhancing inhibitor interaction it is a subtle one however, since they are not strong enough to be identified in the regression analyses.

CONCLUSION

A series of glucosamines designed to explore binding interactions in the region adjacent to the hexokinase glucose binding site have been synthesized and tested. The data obtained was used to develop quantitative structure-activity relationships which demonstrate a significant and specific steric effect as solely responsible for changes in inhibitor potency with changes in inhibitor structure. Molecular modeling of the glucosamine analogs bound in the yeast hexokinase substrate site provided further support for th quantitative analyses as well as illuminating probable causes for the observed low activity of the several congeners that could not be included in the regression equations.

Acknowledgements

This research was supported by National Institutes of Health Research Grant CA45159 (E.A.C. and K.A.S.). The authors wish to thank Mr. H.P. Cordes and Dr. U. Zähringer (Borstel Research Institute) for NMR spectra. Able technical assistance was provided by Mr. David Hock and Ms. Diane Hymel.

References

1. Maley, F. and Lardy, H.A. (1955) *J. Biol. Chem.*, **214**, 765.
2. Steitz, T.A., Anderson, W.F., Fletterick, R.J. and Anderson, C.M. (1977) *J. Biol. Chem.*, **252**, 4494.
3. Anderson, C.M., Stenkamp, R.E., McDonald, R.C. and Steitz, T.A. (1978) *J. Mol. Biol.*, **123**, 207.
4. Easterby, J.S. and Qadri, S.S. (1982) *Meth. Enzymol.*, **90**, 11.
5. Lutz, R.A., Bull, C. and Rodbard, D. (1986) *Enzyme*, **36**, 197.
6. Chandrasekar, R. and Venkatasubramanian, N. (1982) *J. Chem. Soc. Perkin Trans.*, **2**, 1625.
7. Allinger, N.L. (1977) *J. Am. Chem. Soc.*, **99**, 8127.
8. PCMODEL (MMX87), Serena Software, Bloomington, Indiana 1987.
9. Potenzone, R., Cavicchi, E., Weintraub, H.J.R. and Hopfinger, A.J. (1977) *J. Comp. Chem.*, **1**, 187.
10. CAMSEQM/CAMSEQPC, Weintraub Software Design Associates, Cincinnati, Ohio, 1988.
11. Entry 2YHX, version of Jan. 1991.
12. Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F. Jr., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) *J. Mol. Biol.*, **112**, 535.
13. Abola, E.E., Bernstein, F.C., Bryant, S.H., Koetzle, T.F. and Weng, J. (1987) In *Crystallographic Databases—Information Content, Software Systems, Scientific Applications* (F.H. Allen, G. Bergerhoff and R. Sievers, eds.). Bonn/Cambridge/Chester: Data Commission of the International Union of Crystallography, pp. 107–132.
14. SYBYL, Version 5.41, Tripos Associates, Inc., St. Louis, Missouri, 1991.
15. Mukherjee, H. and Pal, P.R. (1970) *J. Org. Chem.*, **35**, 2042.
16. Weininger, D., Weininger, A. and Leo, A.J. (1988) MEDCHEM Software Release 3.53, Daylight Software, Claremont, California.
17. Hansch, C. and Leo, A.J. (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*. New York: John Wiley and Sons.
18. Verloop, A., Hoogenstraaten, W. and Tipker, J. (1976) In *Drug Design* (E.J. Ariens, ed.). New York: Academic Press, Vol. VII, pp. 165–207.