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A theoretical study of glucosamine synthase

Part I.: Molecular mechanics calculations on substrate binding

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Abstract. Glucosamine synthase transfers the γ-amino group of glutamine to fructose, producing 1-glucosamine which is the key constituent of bacterial and fungal cell walls. In this study, model calculations were performed on substrate binding to the enzyme active site. Two models of the active site of glucosamine synthase were proposed, which assume two different sequences of aminoacids, Cys-Gly-Ile and Cys-Ala-Cys, the first one being the *N*-terminal sequence of the *Escherichia coli* enzyme. Several initial geometries were assumed for these tripeptides, the energy was then optimized by means of molecular mechanics. It has been found that the structure which is both energy optimal and satisfies the assumed cysteine sulphur arrangement consists of combinations of C^7_{eq} and C^7_{ax} conformations of single residues. Molecular mechanics calculations were then performed on glutamine and D-fructose-6-phosphate, which are the substrates of the enzymatic catalysis, and on their complex with the enzyme glutamine-binding site. The spatial configuration of the compounds under study, which is optimal as far as the reaction path is concerned, also turned out to be an energy minimum.

Key words: Glucosamine synthase, molecular mechanics, enzymatic catalysis, cell wall biosynthesis, fructose-6-phosphate

Introduction

Glucosamine synthase [Glucosamine-6-phosphate synthase (L-glutamine; D-fructose-6-phosphate amidotransferase (hexose isomerizing EC 2.6.1.16)] transfers the NH_2 group of glutamine to fructose-6-phosphate in an irreversible manner (Winterburn and Phelps 1973). Glucosamine synthase is a key enzyme which belongs to the family of eleven amidotransferases utilising glutamine as a donor of the γ-amino group and transferring it to another substrate (Buchanan 1973). This enzyme catalyses the reaction:

L-glutamine + D-fructose-6-phosphate →
→ D-glucosamine-6-phosphate + L-glutamic acid

The enzyme has been found and isolated from bacteria (Gosh et al. 1960; Kornfeld 1967) and mammalian tissues (Kornfeld 1967; Wintersburn and Phelps 1971) and from some eucaryotic microorganisms such as *Neurospora crassa* (Gosh et al. 1960), *Blastocladiella emersoni* (Norrman et al. 1973) and baker's yeast (Moriguchi et al. 1980).

Owing to its importance in the metabolism of fungal cells, which is explicitly connected with antifungal drug design, numerous experimental studies have been carried out on the structure, mechanism of action and, in particular, inhibitors of glucosamine synthase and other enzymes of this family (Buchanan 1973). All of these studies have revealed that the active site contains cysteine whose sulfhydryl group plays the key role in transferring the NH_2 group from the amido carbon to D-fructose-6-phosphate. The sequence of the aminoacids of the enzyme of the pathogenic fungus *Candida albicans* is still unknown whereas it has recently been determined for *Escherichia coli* bacterium (Badet et al. 1987). In the case of the latter bacterium the amino acid sequence has been found to begin with Cys-Gly-Ile, and the total number of amino acids in the sequence has been estimated to be 616.

Most information about the nature of enzymatic catalysis by glucosamine synthase is supplied by inhibition studies. A number of glutamine derivatives are known to bind irreversibly to the active site of this enzyme. The study of Badet et al. (1987) performed with the use of 6-diazo-5-oxo-L-norleucine (DON) has shown that *Escherichia coli* glucosamine synthase retains half-of-the-sites reactivity when incubated with DON in the absence of D-fructose-6-phosphate, while inactivation in the presence of this sugar is accompa-

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nied by the incorporation of one equivalent of the inhibitor per enzyme subunit. Based on this, a dimeric structure has tentatively been assigned to the enzyme. The very recent study of Badet et al. (1988) in which N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropionic acid (FMDP) was used as inhibitor has demonstrated that this compound is covalently incorporated into the enzyme through the direct addition of the thiol nucleophile to FMDP acting as a Michael acceptor.

A mechnism for the enzymatic catalysis has been proposed by Chmara et al. (1985) which is based solely on the results of the experimental investigations. According to this mechanism, the first stage of catalysis involves a proton transfer from the cysteine hydrosulphide group to a basic imidazole nitrogen histidine which is assumed to be in the immediate neighbourhood of cysteine. This very first step would therefore follow the same course as the mechanism of action of papain (Drenth et al. 1976). Afterwards, the negatively charged mercaptate group thus formed was assumed to attack the side chain carbonyl carbon of glutamine and at the same time the amino group of the glutamine side chain to become detatched from the carbonyl carbon and attack, nucleophilically, the carbonyl carbon of fructose which was assumed to enter into the reaction in its *keto*-form. As a result of this, a thioester and "fructose ammoniate" would be formed. The thioester would then be hydrolysed to restore the cysteine residue with the simultaneous formation of glutamic acid, while the second intermediate, after some simple proton transfers and rearrangements, would give the second product, i.e. glucosamine-6-phosphate.

Although the mechanism of Chmara et al. (1989) is sound and correlates with our knowledge of the action of cysteine proteases which also contain the cysteine S-H groups as active elements, it should be born in mind that owing to the difficulties in obtaining the crystal structure of glucosamine synthase it is hardly possible to assess a *priori* the correctness of any model. The theoretical calculations on both the hypothetical structures of the active center(s) and the reaction path are therefore of vital importance in the subject.

A preliminary CNDO/2 study has been carried out by Tempczyk and Dauter (1985). It has revealed that binding of cysteine sulphur to the glutamine side chain carbonyl carbon does not, in fact, necessitate the prior removal of the sulfhydryl proton. The very first step of the reaction path has been proved to be extremely likely to proceed via a nucleophilic attack of the cysteine sulphur on the side chain carbonyl carbon of cysteine and simultaneous breaking of the S–H bond and binding of the sulfhydryl proton to the amide nitrogen. CNDO/2 gave almost no energy barrier along the corresponding reaction path.

Except for the one mentioned above, no other theoretical studies have been performed on the mechanism of the action of amidotransferases in general and on that of glucosamine synthase in particular. Our present investigations are aimed at continuing the study of Tempczyk and Dauter (1985) in a more detailed and extended way,as well as at designing theoretically the necessary structure of the inhibitors and elaborating a model of the mechanism for their action.

In this work, which is the first stage of our investigations, we propose two models of the active site of the enzyme and the structure of the non-covalent complex with the substrates (i.e. glutamine and sugar) whose formation is assumed to be the first step in the catalysis. In the calculations of the geometry we used the molecular mechanics method, an approach which is justifiable as far as the stage investigated is concerned, because there are no changes in the bond network. In fact, many examples of the use of molecular mechanics and dynamics to study the geometry of enzymes-substrate complexes can be found in published work (Fujita et al. 1986), for example Asn-102 elastase (Lesyng and Meyer 1987), lysosyme (Scheraga 1981), phosphate isomerase (Brown and Kollman 1987). In the case of lysosyme the geometry of the complex with the substrate found by means of molecular mechanics has been shown to be very similar to the structure determined by X-ray analysis (Scheraga 1981).

It is clear that complete theoretical studies must also include the intermediate stages of the reactions and the products formed. As far as the topology of these stationary points in the energy surface of the enzyme-reactant system are known, molecular mechanics can be used to calculate the geometry. However, to establish the direction of the chemical reactions taking place and to evaluate their energetic

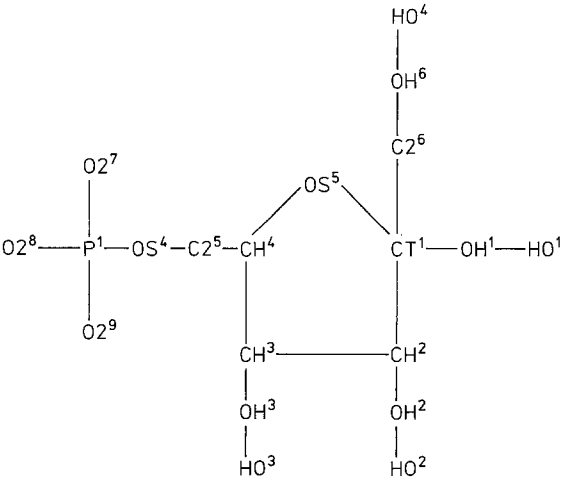

Fig. 1. Molecular mechanics atom types and numbering of atoms of fructose-6-phosphate

effects at least semi-empirical quantum mechanics methods are needed. These calculations are now being carried out in our laboratory.

Methods

In our calculations we have used the force field of Weiner (1984), which is designed for peptides and nucleotides. The united-atom approximation is applied and all gegrees of freedom are assumed to be relaxed. The steric energy is to be expressed by (1).

$$E=\sum_{\text{bonds}} k_d^i(d_i-d_i^\circ)^2+\sum_{\text{bond angles}} k_\theta^i(\theta_i-\theta_i^\circ)^2$$
$$+\sum_{i<j} q_i q_j/\varepsilon r_{ij}+\sum_{\substack{i<j\\ \text{except for H-bonds}}} \varepsilon_{ij}[(r_{ij}^\circ/r_{ij})^{12}-2(r_{ij}^\circ/r_{ij})^6]$$
$$+\sum_{\substack{i<j\\ \text{H-bonds}}} (C_{ij}/r_{ij}^{12}-D_{ij}/r_{ij}^{10})+\sum_{\text{torsion angles}} [\tfrac{1}{2}V_i^1\cos(\omega_i)$$
$$-\tfrac{1}{2}V_i^2\cos(2\omega_i)+\tfrac{1}{2}V_i^3\cos(3\omega_i)]$$
$$\varepsilon_{ij}=(\varepsilon_i\varepsilon_j)^{1/2},\quad r_{ij}^\circ=\tfrac{1}{2}(r_i^\circ+r_j^\circ),$$

where d° and θ° denote strainless values of the bond lengths and bond angles, k_d and k_θ are the respective force constants, q_i is the charge on i-th atom, r_{ij} is the distance between i-th and j-th atom, ε_i and r_i° are the Van der Waals well-depth and radius of atom i; V^1, V^2, and V^3 are the torsional constants.

According to the procedure of Weiner et al. (1984) we scaled down the 1, 4 electrostatic and VDW interaction energies by a factor of 0.5. To perform the calculations we used our previous modification (Liwo et al. 1988) of Allinger's MM2 program (Allinger and Yuh 1977) with the second-order Davidon-Fletcher-Powell minimization procedure (Byrne and Hall 1970).

All the molecular mechanics parameters have been taken from the work of Weiner et al. (1984), except for some charges for the fructose which have been estimated by extrapolation, based on the charges of pentoses given in Weiner's paper. These charges are shown in Table 1, the numbering system for atoms is displayed in Fig. 1.

Results and Discussion

Choosing the model

According to the study of Tempczyk and Dauter (1985), the spatial arrangement of the reactants is very likely to be as shown in Fig. 2 (only the groups directly involved in the reaction and their nearest neighbourhood are indicated for the sake of clarity).

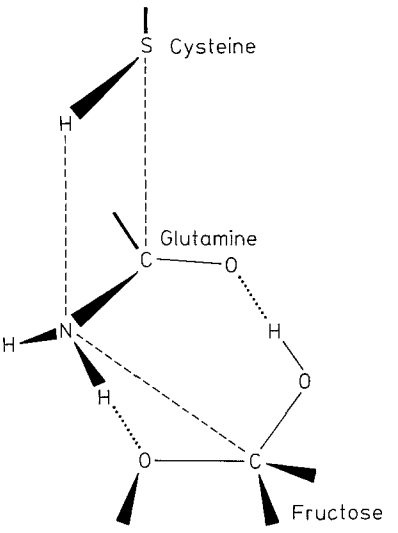

Fig. 2. Schematic representation of the proposed structure of the enzyme-substrate complex. *Broken lines* indicate the assumed path of the reaction, while the *dotted lines* stand for H-bonds assumed to stabilize the structure

Table 1. Charges of fructose-6-phosphate used in molecular mechanics calculations (the atomes are numbered as in Fig. 1)

Atom	Charge (e)	Atom	Charge (e)
CT(1)	0.100	OH(1)	−0.512
CH(2)	0.082	OH(2)	−0.512
CH(3)	0.082	OH(3)	−0.512
CH(4)	0.183	OS(4)	−0.535
C2(5)	0.175	OS(5)	−0.413
C2(6)	0.175	OH(6)	−0.425
		O2(7)	−0.850
		O2(8)	−0.850
		O2(9)	−0.850
Atom	Charge (e)	Atom	Charge (e)
HO(1)	0.316	P(1)	1.429
HO(2)	0.316		
HO(3)	0.316		
HO(4)	0.316		

As is shown, the hydrosulphide group is exactly over the plane of the carboxyamide group of glutamine, the proton facing the lone pair of nitrogen, whereas the negatively charged and easily polarizable sulphur faces the electrophilic carbonyl carbon. This is facilitated by the fact that the C–N and S–H bonds are of almost equal length (about 1.33 and 1.34 Å, respectively). Owing to such an arrangement a new N–H and a new S–C bond can be formed on decreasing the distance between the two groups, with simultaneous braking of the S–H bond. As has been mentioned in the Introduction, in contrast to both the mechanism of Chmara et al. (1985) and the mechanism of action of serine proteases (Drenth et al. 1976) no prior removal of the sulfhydryl proton is required.

Afterwards the C–N bond is likely to weaken and, eventually break, as in the case of serine protease action, the nucleophilic nitrogen at the same time attacking the C^1 sugar carbon (see Fig. 1 for atom numbering) which would initialize the amino group transferring to the sugar molecule. The fructose molecule is, in turn, kept close owing to the presence of two hydrogen bonds indicated in the figure. It can also be presumed that owing to the convenient spatial arrangement of the reacting species all the steps can take place simultaneously, which is very desirable from the kinetic point of view. This assumption is also confirmed by the fact that the enzymatic catalysis considered needs no additional source of energy such as ATP (Gosh et al. 1960).

Owing to the dimeric structure of the *Escherichia coli* enzyme and the tetrameric structure of some other enzymes of the group (Buchanan 1973), it is reasonable to assume that at least two active centers act at any time. This conclusion is supported by the results of the kinetic studies in which DON and fumaroic acids derivatives were used (Badet et al. 1987, 1988; Chmara et al. 1985) which show that the two active centers of the dimeric enzyme are not independent of each other, a condition which can be achieved only if they are geometrically close to each other. Moreover, with such an assumption, the binding to the fumaroic acid derivatives can easily be explained in terms of our proposed mechanism as, due to the presence of the fumaroic acid unit, the inhibitors contain two carbonyl groups with a nucleophilic substituent each, which enables strong interactions both with sulphur and the sulfhydryl proton. From the planarity of the fumaroic acid residue it can also be concluded that the S–H bonds should be able to assume a nearly planar configuration to allow simultaneous binding. This involves, in turn, the near *syn*-configuration of the C–S bonds of the dimer.

In the case of the *Escherichia coli* glucosamine synthase the proximity of the thiol groups must be a result of purely non-bonded interactions between two subunits, as no cysteine residue except for the one in position 3 occurs in the sequence (Badet et al. 1987). However, the possibility must still be allowed for that that glucosamine synthase of some other microorganisms whose structure is yet unknown has two enzyme subunits bonded *covalently* in such a way that the cysteines are also close in the amino acid sequence. The question which immediately arises concerns the number of residues between the cysteines. It is rather unlikely that the cysteines are consecutive, because it would make the two sulphur atoms too close to each other and reactions would occur with difficulty owing to seric hindrance. On the other hand, separating the cysteines by at least two intermediate residues makes the formation of a disulphide bond very probable (Spasov and Popov 1981), which would take away the sulfhydryl protons needed in the reaction (see Fig. 2). It is therefore easy to see that the simplest reasonable model of our hypothetical "covalent active site dimer" involves the Cys-Ala-Cys sequence, as alanine is the simplest chiral amino acid. Because it is very likely that such a sequence is an inner part of the hypothetical covalent dimer, we thought it justifiable to carry out calculations on its terminally blocked form, i.e. Ac-Cys-Ala-Cys-NHMe.

Our second model of the active site assumes the sequence of the first three amino acids of the enzyme isolated from *Escherichia coli,* i.e. Cys^1-Gly^2-Ile^3, not including in the present preliminary calculations two such sequences at a time. Because there is no direct evidence as two whether the enzyme exists in the zwitterionic form or is terminally blocked in some way, we thought it safer to assume a *N*-terminally blocked form (the *C*-terminus of the sequence is obligatorily blocked owing to the continuation of the peptide chain). Therefore the calculations were carried out on Ac-Cys-Gly-Ile-NHMe.

In the following subsections the results of molecular mechanics calculations on the two proposed models of the active site, on substrates of the reactions and on their non-covalent complexes are described in detail.

The hypothetical sequence Ac-Cys-Ala-Cys-NHMe

The calculations in this section were aimed at obtaining such low-energy conformations of the hypothetical active site model for which the S···S distance is not too large and the C–S bonds are nearly parallel. The last two requirements are a direct consequence of the considerations of the preceding section. Owing to the very short length of the backbone fragment we thought it reasonable to search the starting geometries first among the combinations of the lowest-energy conformations of one blocked residue. It must, however, be born in mind that because of the short length of the peptide chain the energetic relations between its conformation need not be the same as when the complete sequence is considered.

For the *N*-acetyl-*N'*-methyl derivatives of most amino acids in aprotic solvents the structure of the greatest population has been experimentally proved to be the "folded" C_{eq}^7 conformation which is characterized by a 1–7 hydrogen bond between the *N*-terminal acetyl carbonyl and *C*-terminal amide hydrogen (Siemion and Picur 1987, 1988). Moreover, both ab initio (Scardsale et al. 1983) and molecular mechanics calculations (Vásquez et al. 1983; Weiner et al. 1984; Zimmerman et al. 1977) indicate unambiguously the energetic preference of this conformation, as far as

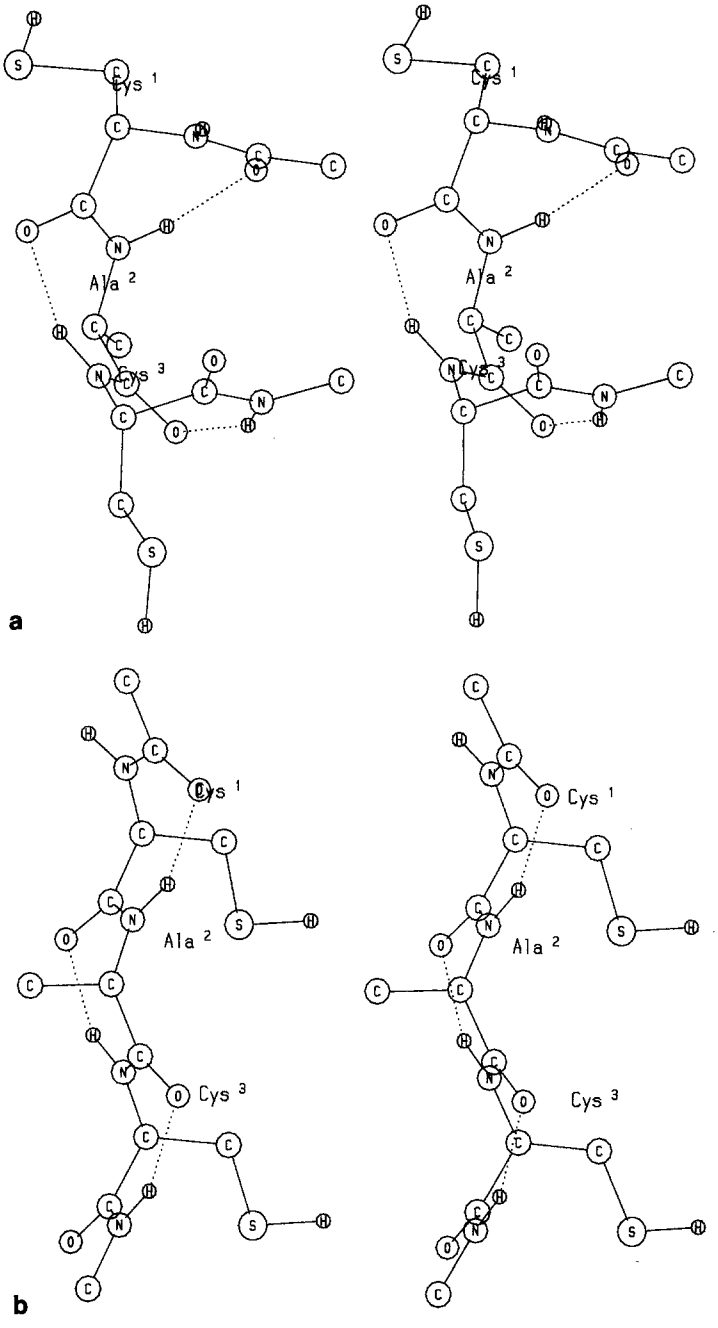

Fig. 3 a and b. The lowest energy (**a**) and used in further calculations (**b**) conformation of the Cys-Ala-Cys fragment

one blocked amino acid residue is concerned. Moreover, the C_{eq}^7 conformation has the same characteristics of the φ and ψ angles, as the top amino acid residue of a γ-turn (Némethy and Printz 1972). The force field of Weiner used in this work gives $\varphi = -76°$, $\psi = 66°$ for alanine. It can also be noted that the second structure with a 1–7 H-bond, the so-called C_{ax}^7 conformation (Zimmerman et al. 1977), can be obtained by reversing the signs of these torsion angles, although its energetic stability depends more on the method applied to the energy evaluation. Nevertheless, as far as Weiner's force field is concerned, it is the second stable conformation and has $\varphi = 68°$, $\psi = -58°$ for alanine. By analogy with the conformation of the γ-turn top amino acid residue, the C_{eq}^7 conformation will further be called a "+" and the C_{ax}^7 conformation a "−" γ-turn.

For the reasons presented above, we first considered the combinations of the "+" and "−" γ-turns. In the case of the central alanine residue the choice of such conformations was also justified by the fact that if our assumption about the structure of the "covalent active site dimer" proves to be true, it is very likely that reversal of the peptide chain occurs at the residue separating cysteines – as in the case of the "top" residue in a "classical" γ-turn (Némethy and Printz 1972).

For a terminally blocked chain composed of three residues there are $2^3 = 8$ different structures containing three γ-turns. Taking into account the sulphur atom arrangement, we can predict that the most suitable are the sequences + + +; − − −; + − + and − + −. Apart from these just check the energy relations, we also examined the remaining sequences, for which the positions of sulphurs are not so convenient. To make the calculations more complete, we also assumed the starting geometries containing type I, type II and type III β-turns at Cys^1–Ala^2 and Ala^2–Cys^3, as turns of this type have been proved to occur very frequently in peptides and proteins (Scheraga 1981), as well as one structure with a 1–13 hydrogen bond between the blocking groups.

Selected torsion angles of those final conformations whose energy is within 3 kcal/mol are summarized in Table 1. As shown, the energy differences between these lowest energy conformations are small. All the low-energy conformations are composed of γ-turns; the ones containing β-turns turned out be relatively high in energy.

The lowest energy conformation is displayed in Fig. 3 a in which the hydrogen bonds are also indicated. As shown, the geometry of the sulfhydryl groups is, however, not convenient in this case. The first conformation which possesses the required sulphur arrangement has a relative energy of 0.9 kcal/mol and is composed of the "−" γ-turns. It is displayed in Fig. 3 b. Clearly, it was the one used in further calculations of the model of the enzyme-substrate complex.

It must be stressed once more that, although in the present calculations the conformations with γ-turns have been assessed to be energy-optimal, this may be a result of considering onyl the first three amino acids out of the whole sequence and neglecting interactions with other constituents of the enzyme. Nevertheless, the results obtained are a reasonable basis for modelling the enzyme-substrate complex.

Table 2. Selected torsion angles of the conformations of the blocked Cys-Ala-Cys

Energy [kcal/mol]		0.0	0.8	0.9	1.5	3.0
Cys^1	φ	71	−77	71	69	−78
	ψ	−62	80	−60	−59	77
	χ^1	179	−174	178	178	−174
	χ^2	−177	−173	−178	−173	−179
Ala^2	φ	−78	−75	69	68	−76
	ψ	65	68	−57	−55	62
Cys^3	φ	69	71	70	−77	−78
	ψ	−60	−58	−59	71	74
	χ^1	−180	179	180	−171	−172
	χ^2	−180	−179	−178	179	−179
S–S distance [Å]		9.97	5.95	5.49	6.71	5.62

Table 3. Selected torsion angles of the conformations of the blocked Cys-Gly-Ile

Energy [kcal/mol]		0.0	1.6	2.3	2.9
Cys^1	φ	71	69	−75	−77
	ψ	−51	−65	81	78
	χ^1	−178	180	−175	−173
	χ^2	−161	−177	−178	−178
Gly^2	φ	82	−78	78	78
	ψ	−52	63	−59	−60
Ile^3	φ	−76	71	−76	73
	ψ	72	−63	75	−61
	χ^1	−180	−55	−51	−54
	χ^2	−168	168	168	167

The Escherichia coli enzyme N-terminal sequence

Ac-Cys-Gly-Ile-NHMe. As in the case of Ac-Cys-Ala-Cys-NHMe we examined the geometries containing all the possible γ-turns and those containing type I, II, and III β-turns. Selected dihedral angles of the conformations whose relative energy is within 3 kcal/mol are in Table 3. The lowest energy conformation is composed of "− − +" γ-turns. It is displayed in Fig. 4 a. As shown, the sulfhydryl group is, however, directed towards the peptide chain, which is reflected in the H-bonds formed. We therefore thought it more justifiable to perform further calculations on the next conformation, for which the sulfhydryl group is positioned outside the chain. It is displayed in Fig. 4 b and is composed of "− + −" γ-turns.

Fructose-6-phosphate. We have energy refined both α- and β-forms of D-fructose-6-phosphate. The β-form turned out to be 3.1 kcal/mol lower in energy, and was the one used in further calculations. The conformations obtained are shown in Fig. 5.

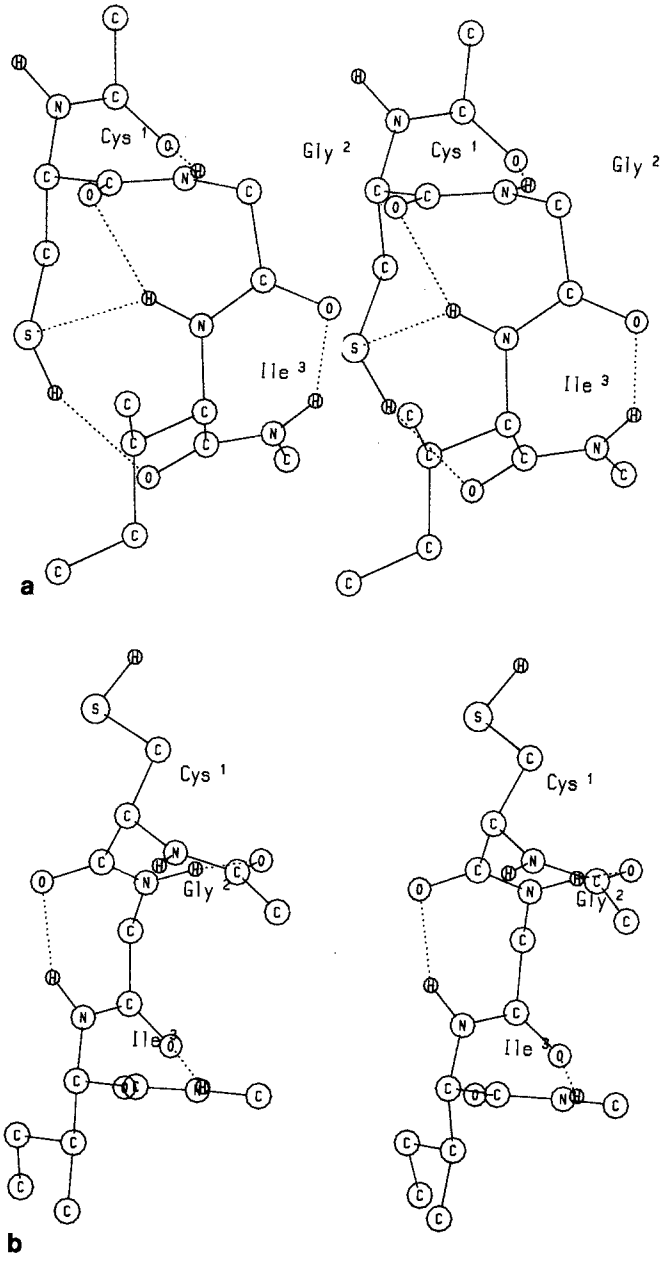

Fig. 4 a and b. The lowest-energy (**a**) and used in further calculations (**b**) conformation of the Cys-Gly-Ile fragment

Glutamine. The glutamine involved in the reaction is probably supplied as part of a larger peptide (Chmara et al. 1985). Therefore in the calculations we have assumed its terminally blocked form. It is most probable that in the reaction the glutamine moiety occurs in its extended form, both in the case of the backbone and the side chain. The starting conformation was obtained using the crystallographic data of extended glutamine as a starting point in the energy minimization (Koetzle et al. 1973). The final conformation is displayed in Fig. 5.

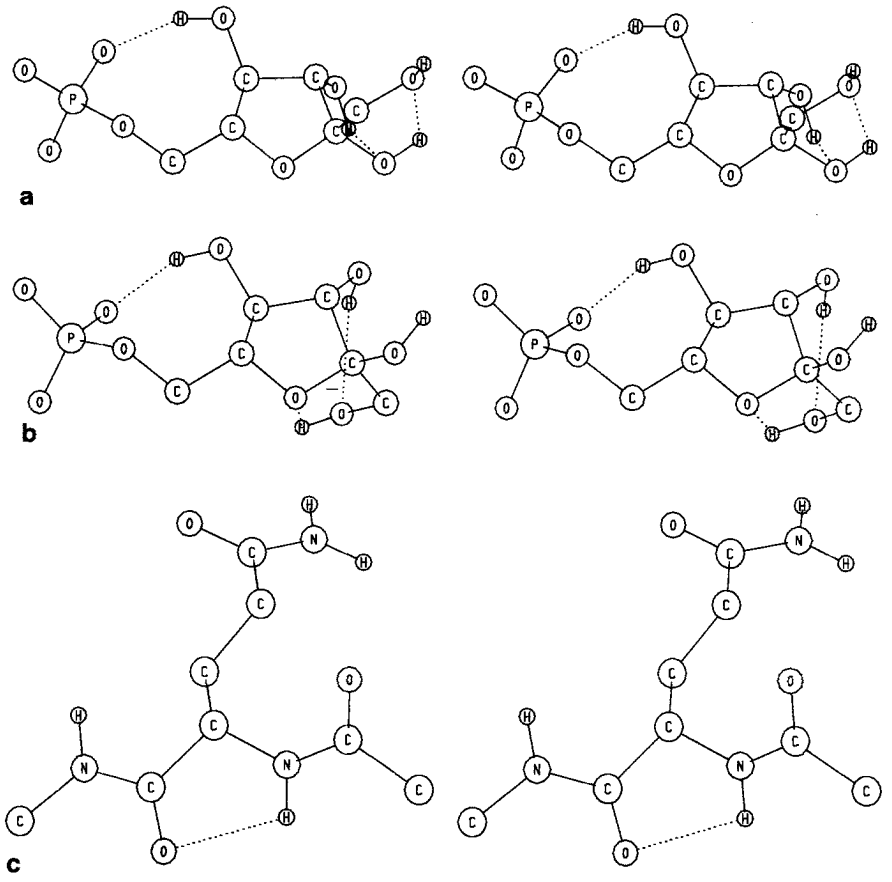

Fig. 5 a–c. Energy-optimized geometries of β- (**a**) and α- (**b**) forms of fructose-6-phosphate and glutamine (**c**)

The models of the enzyme-substrate complex. The last step of this study was to calculate the equilibrium geometry of the complex of the enzyme active site models with the substrates, i. e. fructose-6-phosphate and blocked glutamine. As indicated above, we used both the hypothetical sequence (Ac-Cys-Ala-Cys-NHMe) and the *N*-terminal sequence of the *Escherichia coli* (Ac-Cys-Gly-Ile-NHMe) whose conformations have been described in the preceding sections. In each case we assumed the binding of one glutamine and one sugar molecule per active site. We assumed such an initial spatial arrangement of the components of the complex which meets the reaction center geometry of Fig. 2. In the case of Ac-Cys-Ala-Cys-NHMe it was a question of which sulfhydryl group should be chosen to interact with glutamine carboxyamide. Based on the results of the normal mode analysis performed for the tripeptide we have concluded that the *C*-terminal cysteine sulphur is more labile, as the energy of the modes involving the rotation about the $C_\alpha - CH_2$ bond is lower in this case than for the *N*-terminal cysteine. Thus, in establishing the starting geometries we located the S–H group of the *N*-terminal cysteine over the glutamine carboxyamide.

Since we have found the charged phosphate group interactions to be very strong when compared with those involving other electronegative groups of the systems examined, we have substituted it by a methyl group. On the other hand, it is known (Litowska and Tempczyk 1987), that the phosphate group is in a very rigid environment owing to the interactions with other phosphate groups of the cell wall's teichoic acids and has therefore no chance of markedly changing its position on interaction with other molecules. Thus in our case the role of the interactions involving the phosphate group would, at any rate, be exaggerated. Besides, in our model the phosphate group is presumed not to take part in this reaction.

We have examined several initial geometries of the complex in the case of Ac-Cys-Ala-Cys-NHMe and one in the case of Ac-Cys-Gly-Ile-NHMe. Apart from the structures with the hydrogen bond between fructose H^1 and glutamine side chain carbonyl we have also considered the structures in which this H-bond is replaced by the one between the fructose H^4 and glutamine side chain carbonyl. However, such an assumption gave a significantly higher energy value.

The lowest energy conformations of Ac-Cys-Ala-Cys-NHMe and Ac-Cys-Gly-Ile-NHMe obtained as a result of energy minimization are shown in Figs. 6 and 7, respectively. The hydrogen bonds and the assumed directions of the reaction are also indicated in the fig-

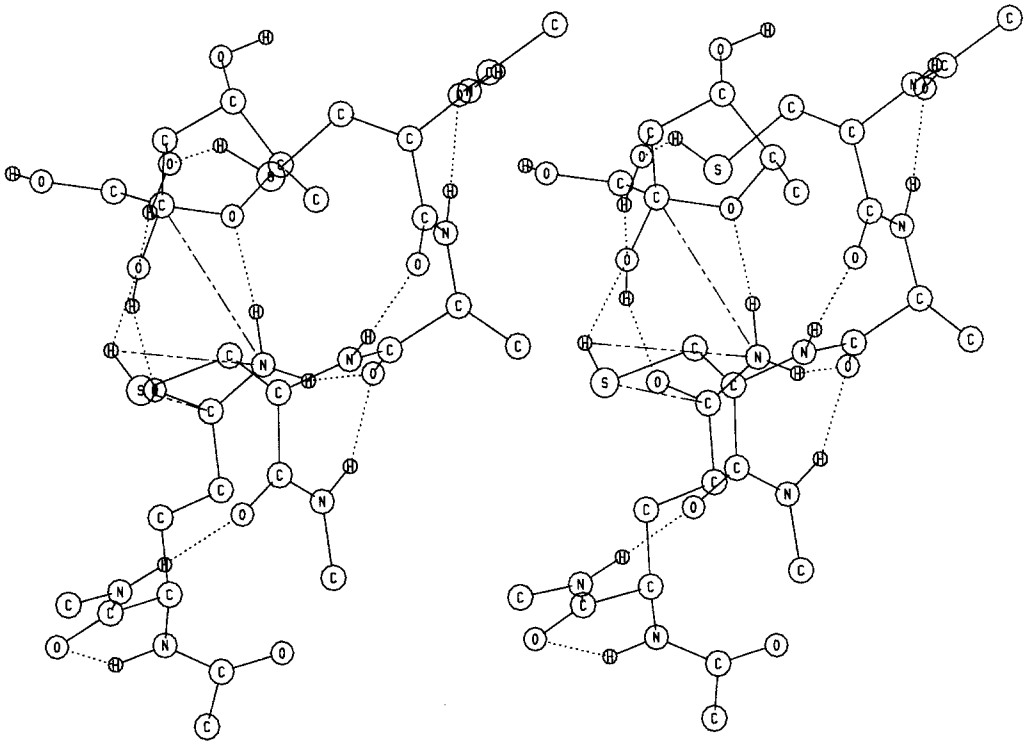

Fig. 6. The lowest-energy structure of the complex of the Cys-Ala-Cys fragment with glutamine and D-fructoso-6-phosphate. Hydrogen bonds are indicated by *dotted lines*, while *broken lines* show the assume path of the first step of the reaction (cf. Fig. 2)

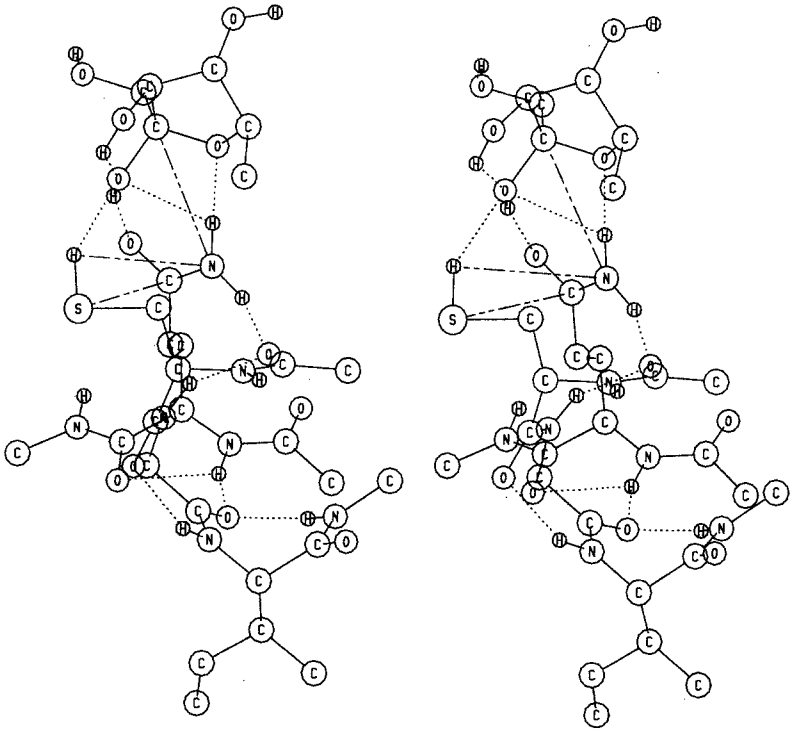

Fig. 7. The lowest-energy structure of the complex of the Cys-Gly-Ile fragment with glutamine and sugar

ures. The interatomic distances of particular interest (cf. Fig. 2) and the energy gains on substrate binding are in Table 4.

As is shown, although the proposed reaction center geometry has become a little distorted from the "ideal" arrangement shown in Fig. 2, it has not changed qualitatively. In particular hydrogen bonds have remained between fructose O^5 and glutamine side chain amide proton and between fructose H^1 and glutamine side chain carbonyl oxygen. Moreover the

Table 4. Selected interatomic distances of the lowest energy structure of the complex of Ac-Cys-Ala-Cys-NHMe with substrates

Groups involved [a]		Interatomic distance [Å]	
		Cys-Ala-Cys	Cys-Gly-Ile
S (Cys)	$\cdots C''$ (Gln)	3.87	4.23
S (Cys)–H	$\cdots N''$ (Gln)	3.75	3.76
N'' (Gln)	$\cdots C^1$ (Fru)	3.52	3.46
N''–H (Gln)	$\cdots O^5$ (Fru)	1.87	1.82
O^1–H^1 (Fru)	$\cdots$ O–C″ (Gln)	1.81	1.84
Energy gain [kcal/mol]		−35.4	−28.8

[a] The symbol ″ labels the glutamine side chain atoms

"ideal" geometry can easily be reached owing to the thermal motion. This is very important, because it indicates that the energetic factor will act so as to keep the reactants in the position from which the reaction is likely to start. Besides, we must not forget that because only short fragments of the peptide chains have been considered in this study, the components are, in fact, fully flexible and the forces which hold the substrates in the real enzyme can be much stronger. Anyway, even in this very simplified model we observe considerable energy gains on substrate binding: −35.4 kcal/mol for the complex with Ac-Cys-Ala-Cys-NHMe and −28.8 kcal/mol for Ac-Cys-Gly-Ile-NHMe which may itself be responsible for the formation of the enzymatic hole [note that the energy dispersion per protein molecule is about 42 kcal/mol (Lesyng and Meyer 1988)]. The greater energetic effect in the first case is directly connected with the presence of the second hydrophilic cysteine side chain which is also reflected in additional H-bonds formed with the sulfhydryl group of this cysteine.

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