CONFORMATIONAL ANALYSIS OF GLYCOPROTEINS

Part I. Conformation of the Protein Segment at the Site of Peptide-sugar Linkage

M. NAGARAJAN AND V. S. R. RAO

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012

ABSTRACT

The prediction of the secondary structure of about 55 Glycoproteins which contain Asn (CHO)–X–Ser/Thr, Asp (CHO)–X–Ser/Thr, Ser(CHO)–X–Y, and Thr(CHO)–X–Y was carried out by using the methods of Chou and Fasman. In most of the cases amino acid sequence at the site of sugar linkage assumes a β-turn. The present analysis also suggests that the nature of the amino acid residue X [Asn(CHO)–X–Ser/Thr, Asp(CHO)–X–Ser/Thr, Ser(CHO)–X–Y and Thr(CHO)–X–Y] plays a major role in deciding whether all or part of the amino acid residues in these sequences are involved in the β-turn. In Aspartic acid linked sugars, the triplet sequence Asp(CHO)–X–Ser/Thr is conserved [like in Asn(CHO)–X–Ser/Thr] and bend begins with Aspartic acid (though such examples are very few), thus involving the complete triplet sequence in the formation of the β-turn. In proteins which contain Ser(CHO)–X–Y and Thr(CHO)–X–Y sequences, the amino acid residue preceding the above sequences is generally either Pro or Gly. These results are in disagreement with the 'code sequence'. Thr/Ser (CHO)–X–Y–Pro– proposed for the linkage of sugar to Threonine or Serine from the earlier studies.

GLYCOPROTEINS belong to an important group of biopolymers, which occur in connective tissues, blood cells, serum, urine, saliva and other body fluids and serve various biological functions. They consist of essentially a protein backbone to which carbohydrates are linked covalently. They differ not only in the relative proportions of types of sugar but also in the number of side chains present. The frequency of occurrence of oligosaccharides along the peptide chain also varies markedly from one protein to another. Out of the twenty different amino acids, only L-asparagine, L-serine, L-threonine, L-aspartic acid are common protein constituents which form covalent linkages with carbohydrates. In glycoproteins where carbohydrate is linked to asparagine, a common sequence Asn(CHO)-X-Ser(Thr) has been observed. Such sequences are also found in proteins which lack the sugar. The importance of occurrence of Ser(Thr) in the third position has been explained by Marshall and Neuberger[1] who suggested a hydrogen bond between the carbonyl group of the side chain of asparagine and the hydroxyl group of hydroxy amino acid, to decrease the dissociation constant of the amide group, thereby facilitating the attachment of sugar to asparagine residue. However nothing is known about the possible conformation of the protein chain around the site of peptide-carbohydrate linkage and the nature of the amino acid residues. Such information may throw light on the stereochemical requirement at the site carbohydrate-protein linkages.

Method of Calculation: Recently Fasman and Chou[2–4], from the analysis of the 20 naturally occurring amino acids proposed a set of parameters P_α, P_β, P_o and p_t for prediction of protein conformation based on the frequency of occurrence of amino acids in the α-helical, β-sheet, coil and β-turn regions respectively from the known protein structures. These parameters have been used to assign the regions of α and β, coil and β-turn regions. The relative probability p_t of a tetrapeptide to form a β-turn was computed from the frequency of occurrence of each residue at the 1st, 2nd, 3rd and 4th positions[4]. The cut off value of β-turn[4] was chosen as $1{\cdot}6 \times 10^{-4}$. In cases, where the complete sequences are not known, as far as possible, at least three amino acid residues, on either side of an amino acid to which sugar is attached were considered. The amino acid residues that are involved in the β-turn are underlined by the solid line. The average parameters $\langle P_t \rangle$, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ computed for the tetrapeptide involved in the β-turn, following the procedure outlined by Fasman and Chou are also shown in Tables I, II, III and IV. These average values are obtained by adding the individual P_α, P_β and P_t values of the residues in the tetrapeptide under consideration and dividing by four, the number of residues involved.

RESULTS AND DISCUSSION

The amino acid sequences around carbohydrate-peptide linkages of several glycoproteins are displayed in Tables I, II, III and IV. It is seen from Table I that mostly in glycopeptides with GlcNAc-Asn group, the sequence is either Asn-X-Thr or Asn-X-Ser. However very few exceptions have also been observed where the third residue is not a hydroxy amino acid. This indicates that exceptions to the suggestions of Marshall and Neuberger do occur (though rarely) where glycosylation of the asparagine takes place without the presence of hydroxy amino acid, serine or threonine in the third place of the sequence Asn-X-Thr(Ser). It

TABLE I

$\langle P_t \rangle$, $\langle P_\beta \rangle$, $\langle P_\alpha \rangle$ and P_t values of glycopeptides at the site of sugar linkage to asparagine residue

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t \rangle$	$\langle P_\beta \rangle$	$\langle P_\alpha \rangle$
(1)	(2)	(3)	(4)	(5)	(6)
Porcine Luteinising hormone–α-subunit	–Arg–Val–Glx–Asn*–Ser–Thr–Gln[5]	2·20	1·27	0·95	0·88
Follicle stimulating hormone–β-subunit	–Leu–Thr–Ile–Asn*–Thr–Thr–Trp[5]	3·78	1·21	1·06	0·88
β-lipotrophic hormone	–Asp–Arg–Ser–Asn*–Ala–Thr–Leu[5]	2·98	1·34	0·77	0·82
Bovine Prothrombin	–Tyr–Arg–Gly–Asn*–Val–Ser–Val[5]	2·57	1·28	0·91	0·67
	–Pro–Glu–Ile–Asn*–Ser–Thr–Thr	2·20	1·32	0·94	0·79
	–Trp–Asp–Lys–Asn*–Phe–Thr–Val	1·95	1·07	0·87	0·98
Lamprey Fibrinopeptide	–Ala–Thr–Asn–Asn*–Ser–Asp–Pro[5]	3·18	1·43	0·81	0·77
		3·13	1·44	0·70	0·77
Deoxy Ribonuclease A, B, C	–Lys–Met–Ser–Asn*–Ala–Thr–Leu[5]	1·67	1·12	1·00	1·04
Stem Bromelain	–Gln–Ser–Asn–Asn*–Glu–Ser–Ser[b]	2·10	1·32	0·95	0·57
Transferrin	–Leu–Ile–His–Asn*–Arg–Thr–Gly[5]	2·89	1·30	0·72	0·89
Haptoglobin β-chain (human)	–His–Gln–Asn*–Asn–Ser–Thr–Ala[5]	3·66	1·21	0·81	0·97
		2·47	1·43	0·81	0·77
Bovine Thrombin B chain	–Phe–Lys–Asn*–Pro–Thr–Val[5]	1·68	1·12	1·03	0·82
Porcine Pancreatic Lipases L_A and L_B	–Thr–Asn*–Gly–Thr–Ile–[6]	2·34	1·34	0·97	0·73
Human and Porcine Ceruloplasmin	–Ala–Ile–Tyr–Asn*–Asp–Thr–Thr[7]	2·25	1·28	0·99	0·79
Phosvitin	–Ser–Asn*–Ser–Gly[17]	3·28	1·51	0·73	0·71
Phospholipase A[a] (honey bee)	–Gly–His–Gly–Asn*–Lys–Ser–Ser[25]	5·13	1·21	0·73	0·89
		3·16	1·36	0·71	0·85

* denote the site of attachment of sugar residue to the amino acid.
[a] denote the site of sugar linkage is not certain.
[b] numbers on the top of the amino acid sequence denote the references.
[c] the amino acids that are involved in a β-turn are underlined.

is also interesting to note (Table IV) that in general the third amino acid residue is also a hydroxy amino acid, in aspartic acid linked carbohydrate moieties [Asp (CHO)-X-Ser/Thr]. On the other hand that in Ser (CHO)-X-Y, Thr(CHO)-X-Y, Y need not necessarily be a hydroxy amino acid (Tables II and III).

The p_t values of the amino acid sequences (Tables I–IV) at the site of sugar linkage are mostly higher than $1{\cdot}6 \times 10^{-4}$ and the average value of (P_t) is higher than (P_α) and (P_β) suggesting that the peptide chain at the site of sugar linkage assumes a β-turn whether the sugar is linked to Asn, Thr, Ser or Asp. Similar results have

TABLE II

$\langle P_t \rangle$, $\langle P_\beta \rangle$, $\langle P_\alpha \rangle$ and p_t values of glycopeptides at the site of sugar linkage to serine residue

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t \rangle$	$\langle P_\beta \rangle$	$\langle P_\alpha \rangle$
Human Chorionic Gonadotropin-β-subunit	-Ser-Ser-Ser-Ser*-Lys-Ala-Pro[5]	3·16	1·34	0·73	0·86
	-Pro-Pro-Pro-Ser*-Leu-Pro-Ser	4·16	1·18	0·80	0·83
	-Pro-Ser-Pro-Ser*-Arg-Leu-Pro	5·83	1·31	0·74	0·74
	-Pro-Gly-Pro-Ser*-Asp-Thr-Pro	3·79	1·33	0·84	0·80
Hinge region of IgA_1 Myeloma Protein (Pat, Ose)	-Pro-Thr-Pro-Ser*-Pro-Ser-Thr[30]	3·14	1·33	0·79	0·70
Carbohydrate units of IgA_1 Immuno-globulin	-Pro-Thr-Pro-Ser*-Pro-Ser-Thr-	3·14 6·45	1·33 1·36	0·79 0·82	0·70 0·75
	-Pro-Thr-Pro-Ser*-Pro[31]	3·14	1·33	0·79	0·70
Fetuin Glycopeptides	-Gly-Pro-Ser*-Pro-Thr-Ala[21]	3·59 2·12	1·43 1·18	0·69 0·88	0·63 0·91
	-Gly-Ser*-Thr-Pro-Gly	1·22	1·38	0·84	0·68
Disease Protein ZUC	-Pro-Gly-Gly-Ser*-Ser-Glu-Pro[32]	4·21	1·48	0·74	0·61
Bovine submaxillary Mucin	-Ser-Thr*-Gly-Ser-[33]	5·50	1·41	0·86	0·73
α-amylase	-Ser*-Glu-Asp-Gly[34]	2·38	1·28	0·96	0·65

* denote the site of attachment of sugar residue to the amino acid.
[b] numbers on the top of the amino acid sequence denote the references.
[c] the amino acids that are involved in a β-turn are underlined.

also been obtained in other sequences containing Asn-sugar linkage, *i.e.*, Thr-Val-Asn(CHO)-Thr ($p_t = 1{\cdot}65 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}07$, $\langle P_\beta \rangle = 1{\cdot}14$, $\langle P_\alpha \rangle = 0{\cdot}88$); and Pro-Lys-Asn(CHO)-Ile ($p_t = 2{\cdot}72 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}11$, $\langle P_\beta \rangle = 0{\cdot}90$ and $\langle P_\alpha \rangle = 0{\cdot}85$) in hormones[5], Ser-Arg-Asn(CHO)-Leu ($p_t = 3{\cdot}68 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}13$, $\langle P_\beta \rangle = 0{\cdot}87$, $\langle P_\alpha \rangle = 0{\cdot}91$) and Arg-Arg-Asn(CHO)-Met ($p_t = 1{\cdot}69 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}04$, $\langle P_\beta \rangle = 1{\cdot}03$, $\langle P_\alpha \rangle = 0{\cdot}88$) in Ribonucleases[5,11–16], Asn (CHO)-Gln-Thr-Gly ($p_t = 2{\cdot}32 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}29$, $\langle P_\beta \rangle = 0{\cdot}97$, $\langle P_\alpha \rangle = 0{\cdot}81$) in human Lacto-transferrin[8], Ser-Asn(CHO)-Tyr-Ser ($p_t = 2{\cdot}31 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}39$, $\langle P_\beta \rangle = 0{\cdot}85$, $\langle P_\alpha \rangle = 0{\cdot}73$) in Taka Amylase A[9], ASn(CHO)-LYS-Ser-Asp ($p_t = 1{\cdot}90 \times 10^{-4}$); $\langle P_t \rangle = 1{\cdot}33$, $\langle P_\beta \rangle = 0{\cdot}73$, $\langle P_\alpha \rangle = 0{\cdot}89$) in human serotransferrin[10], Pro-Ser-Asn (CHO)-Ser ($p_t = 5{\cdot}79 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}46$, $\langle P_\beta \rangle = 0{\cdot}68$, $\langle P_\alpha \rangle = 0{\cdot}73$) in Guinea pig B Ribonuclease[11], Ser-Ser-Asn(CHO)-Ser ($p_t = 3{\cdot}98 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}49$, $\langle P_\beta \rangle = 0{\cdot}70$, $\langle P_\alpha \rangle = 0{\cdot}78$); and Gln-Ser-Asn(CHO)-Ser ($p_t = 3{\cdot}16 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}35$, $\langle P_\beta \rangle = 0{\cdot}83$, $\langle P_\alpha \rangle = 0{\cdot}87$) in porcine Ribonuclease[14], Ser-Ser-Asn(CHO)-Pro ($p_t = 3{\cdot}84 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}46$, $\langle P_\beta \rangle = 0{\cdot}68$, $\langle P_\alpha \rangle = 0{\cdot}73$) in horse Ribonuclease[16], Met-Asn(CHO)-Gly-Thr ($p_t = 1{\cdot}94 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}24$, $\langle P_\beta \rangle = 1{\cdot}08$, $\langle P_\alpha \rangle = 0{\cdot}82$) in Bovine Visual Pigment 500[18], Gly-Glu-Asn(CHO)-Arg ($p_t = 1{\cdot}59 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}19$, $\langle P_\beta \rangle = 0{\cdot}90$, $\langle P_\alpha \rangle = 0{\cdot}66$) in Fibrinogen[19], Asn(CHO)-Arg-Thr-Gly ($p_t = 2{\cdot}89 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}30$, $\langle P_\beta \rangle = 0{\cdot}72$, $\langle P_\alpha \rangle = 0{\cdot}89$) in hen ovotransferrin[20], Ser-Asn (CHO)-Gly-Ser ($p_t = 3{\cdot}93 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}51$, $\langle P_\beta \rangle = 0{\cdot}71$, $\langle P_\alpha \rangle = 0{\cdot}73$); Asn(CHO)-Asp-Ser-Arg (p_t

TABLE III

$\langle P_t \rangle$, $\langle P_\beta \rangle$, $\langle P_a \rangle$ and p_t values of glycopeptides at the site of sugar linkage to Threonine residue

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t \rangle$	$\langle P_\beta \rangle$	$\langle P_a \rangle$
Rabbit Immunoglobulin G[b,c]	–Ser–Lys–Pro–Thr*–Cys–Pro–Pro[35]	1·83	1·20	0·94	0·69
Phospholipase A (honey bee)[a]	–Tyr–Pro–Gly–Thr*–Leu–Trp–Cys[25]	7·05	1·27	0·83	0·49
Cow χ_A Casein[a]	–Pro–Thr*–Thr–Thr*–Pro–Thr–Thr–[36]	1·83	1·19	1·06	0·76
		2·84	1·19	1·06	0·76
	–Pro–Ser*–Ser–Ser–Pro	3·49	1·44	0·70	0·74
Sheep χ_A Casein[a]	–His–Ser–Thr*–Pro–Thr*–Thr*–Glu–Ala–Val[37]	2·84	1·19	1·06	0·76
Alanine Apolipoprotein (apoC–III)	–Val–Arg–Pro–Thr*–Ser–Ala–Val[38]	2·10	1·23	0·86	0·75
Encephalitogenic Al protein	–Thr–Pro–Arg–Thr*–Pro–Pro–Pro[39]	2·54	1·14	0·98	0·76

* denote the site of attachment of sugar residue to the amino acid.
[a] denote the site of sugar linkage is not certain.
[b] numbers on the top of the amino acid sequence denote the references.
[c] the amino acids that are involved in a β-turn are underlined.

TABLE IV

$\langle P_t \rangle$, $\langle P_\beta \rangle$, $\langle P_a \rangle$ and p_t values of glycopeptides at the site of sugar linkage to aspartic acid

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t \rangle$	$\langle P_\beta \rangle$	$\langle P_a \rangle$
F_c region of Rabbit heavy chain[b,c]	–Gln–Gln–Phe–Asp*–Ser–Thr–Ile[40]	1·92	1·14	1·05	0·90
IgG heavy chain (man baboon, monkey, cow, pig, dog and cat)	–Glx–Glx–Phe–Asp*–Ser–Thr–Tyr[41]	2·98	1·26	1·00	0·80
	–Gly–Glx–Phe–Asp*–Ser–Thr–Ile	1·92	1·14	1·08	0·90
Thrombin B chain (bovine)	–Phe–Lys–Asp*–Pro–Thr–Val[5]	2·01	1·08	1·07	0·88
Protein CRA γ_1	–Asp–Ile–Phe–Asp*–Asp–Arg–Thr[42]	1·91	1·20	0·89	0·93

* denote site of attachment of sugar residue to the amino acid.
[b] numbers on the top of the amino acid sequence denote the references.
[c] the amino acids that are involved in a β-turn are underlined.

$= 2{\cdot}57 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}34, \langle P_\beta \rangle = 0{\cdot}77, \langle P_a \rangle = 0{\cdot}82$), and Asn(CHO)-Asp-Ser-Cys ($p_t = 2{\cdot}35 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}34, \langle P_\beta \rangle = 0{\cdot}82, \langle P_a \rangle = 0{\cdot}87$) in Fetuin glycopeptides[21], Gly-Pro-Asn(CHO)-Gln ($p_t = 8{\cdot}49 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}34$, $\langle P_\beta \rangle = 0{\cdot}83$, $\langle P_a \rangle = 0{\cdot}76$) in human parotid Glycoprotein[22]; Ser-Pro-Asn(CHO)-Ala ($p_t = 5{\cdot}58 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}28$, $\langle P_\beta \rangle = 0{\cdot}74$, $\langle P_a \rangle = 0{\cdot}89$); Asn(CHO)-Thr-Thr-Tyr ($p_t = 2{\cdot}44 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}22$, $\langle P_\beta \rangle = 1{\cdot}09$, $\langle P_a \rangle = 0{\cdot}75$), Asn(CHO)-Arg-Ser-Ser ($p_t = 3{\cdot}83 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}36$, $\langle P_\beta \rangle = 0{\cdot}78$, $\langle P_a \rangle = 0{\cdot}78$), Asn(CHO)-Ser-Thr-Gln ($p_t = 2{\cdot}20 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}27, \langle P_\beta \rangle = 0{\cdot}95, \langle P_a \rangle = 0{\cdot}88$), Asn(CHO)-Thr-Thr-Ser ($p_t = 2{\cdot}55 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}32$, $\langle P_\beta \rangle = 0{\cdot}94$ $\langle P_a \rangle = 0{\cdot}79$) and Tyr-Asn(CHO)-Asn-Ser ($p_t = 3{\cdot}45 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}43, \langle P_\beta \rangle = 0{\cdot}83$, $\langle P_a \rangle = 0{\cdot}72$) in Horse radish Peroxidase[23], Asp-Arg-

Ser Asn(CHO) ($p_t = 2{\cdot}98 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}34$, $\langle P_\beta \rangle = 0{\cdot}77$ $\langle P_\alpha \rangle = 0{\cdot}82$) in Posterior Pituitary (Pig) Glycopeptides[24], Thr-Pro-Asn(CHO)-Lys ($p_t = 7{\cdot}10 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}24$, $\langle P_\beta \rangle = \langle P_\alpha \rangle = 0{\cdot}80$); Asn(CHO)-Ser-Ser-Tyr ($p_t = 3{\cdot}96 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}39$, $\langle P_\beta \rangle = 0{\cdot}85$, $\langle P_\alpha \rangle = 0{\cdot}73$) in α 1 acid glycoprotein[26]; Gly-Ser-Asn (CHO)-Met ($p_t = 3{\cdot}34 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}32$, $\langle P\beta \rangle = 0{\cdot}96$ $\langle P_\alpha \rangle = 0{\cdot}81$) in Egg White Avidin[27]; Asn(CHO)-Lys-Thr-Ser ($p_t = 1{\cdot}99 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}27$, $\langle P_\beta \rangle = 0{\cdot}83$, $\langle P_\alpha \rangle = 0{\cdot}85$) in γ chain of human fibrinogen[28] and Asn(CHO)-Thr-Gly-Val ($p_t = 1{\cdot}94 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}17$, $\langle P_\beta \rangle = 1{\cdot}08$, $\langle P_\alpha \rangle = 0{\cdot}81$) in Mucor Micher Protease[29]. When this manuscript was completed Aubert *et al.*[43] (1976) from similar analysis of 21 glycoproteins pointed out that 19 out of 28 asparagine-sugar linkages and all the 9 Ser/Thr-sugar linkages assume β-turns. On the other hand the present study based on similar analysis of about 55 glycoproteins predicts that 59 out of 61 asparagine-sugar linkages and all the 21 Ser/Thr-sugar linkages are involved in β-turns, thus showing some discrepancy about the conformation of the protein segment around the Asn-sugar linkages. This may be due to the fact that the Aubert *et al.*[43] (1976) have used original values of Chou and Fasman[2,3] for predicting the β-turns, whereas in the present study the revised values of Chou and Fasman[4] have been made use of. The 2 exceptions in the case of Asn-sugar linkages may be in the disordered or β-sheet regions. The attachment of carbohydrate to protein is known to be post-ribosomal[44] and the sugar linkage is on the surface of the glycoproteins[45–47]. It may be considered that the bend may facilitate the exposure of the side chain of the amino acid for the enzyme to mediate glycosilation.

The amino acid residues occurring in the region around the sugar linkage may be broadly classified into β-turn promoters whose $P_t > 1{\cdot}0$ and non-β-turn promoters whose $P_t < 1{\cdot}0$. In the sequence Asn-X-Ser/Thr, if X is a β-turn promoter such as Gly, Asn, Ser, Pro, and Asp, the triplet has enough potential to form a β-turn, for three of the four residues required to form a β-turn, are β-turn promoters. On the other hand, if X is a non-β-turn promoter residue, the following residue after the triplet sequence Asn-X-Ser/Thr or the two or one of the two preceding residues of this triplet should be β-turn promoters, to satisfy the condition that at least three out of four residues should be β-turn promoters[4] in order to form a bend. It seems that if X is not a β-turn promoter (Ala, Leu, Val, Gln, etc.) the nature of the preceding or following residues to the sequence Asn-X-Ser (Thr) also appear to be important to facilitate the attachment of sugar to the protein chain. It is interesting to note that in sequences Asn-X-Ser(Thr) where X is not a β-turn promoter (Ala, Leu, Lys, etc.) depending upon the nature of the preceding residues, the hydroxy amino acid Ser or Thr in the above sequence may or may not be involved in the formation of a β-turn. It is also interesting to note that in proteins which contain Ser(CHO)-X-Y and Thr(CHO)-X-Y sequences (Tables II and III) the amino acid residue preceding the above sequences in the protein is generally either pro or gly (the β-turn promoters). Table IV shows (though the examples are few) that in Asp linked carbohydrates, the β-turn always begins with Asp and all the amino acids in the triplet sequence Asp(CHO)-X-Thr/Ser are involved in the β-turn.

Ser/Thr(CHO)-X-Y-Pro was suggested earlier as 'code sequence'[36] similar to Asn(CHO)-X-Ser/Thr for glycopeptides containing the N-glucosamine linkage. Tables II and III indicate that in the above sequence Ser/Thr(CHO)X–Y–Z, Z need not necessarily be a pro in disagreement with the earlier suggestions.

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For details contact : Prof. S. S. Pawar, Biochemistry Division, Chemistry Department, Marathwada University, Aurangabad 431002.