OCCURRENCE OF PHOSPHORYLATED RESIDUES IN PREDICTED β-TURNS: IMPLICATIONS FOR β-TURN PARTICIPATION IN CONTROL MECHANISMS*

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Summary

Twenty-four out of thirty phosphorylated residues (80%) contained in fourteen different proteins were found to exist within regions predicted as β-turns. Phosphorylated sites not predicted within turns were found to be adjacent to predicted turns (± 2 residues) in four other cases. Two proteins were found to be phosphorylated in regions not associated with β-turns. Thus, β-turns may play a more active role in biological function in addition to its directional effect on the folding of globular proteins.

Introduction

An active area of research has been the role of protein phosphorylation in regulating both metabolic processes and genetic expression (1). The phosphorylation of these proteins takes place at highly specific sites. Williams (2) attempted to correlate primary sequence with sites of phosphorylation, however, sufficient sequence homologies do not exist to explain this specificity. The possible recognition of a specific secondary structure by the phosphorylating enzyme could possibly offer an attractive explanation.

β-turns are a type of secondary protein structure composed of four amino acid residues arranged in such a manner as to facilitate chain reversal (3). Due to the nature of this role, β-turns are located predominantly at the surfaces of proteins, where the polypeptide backbone meets its globular boundary. Thus, a β-turn would be propitiously situated to mediate these effects: being a secondary structure that might be recognizable and accessible to a phosphorylating enzyme. The chemical modification could have its effect by providing interactions either favorable to sub-unit association or disassociation. There is the additional possibility that in analogy to the role of glycosylation in sickle cell structure (4), phosphorylation could cause a change in conformation mediated through the β-turn.

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Table 1

Prediction of Phosphorylated Residues Occurring Within β-turns Using Different p_t Cut-off Values

p_t cut-off value	Number of phosphorylated residues predicted as occurring within β-turns
1.0×10^{-4}	19/30
0.9×10^{-4}	22/30
0.8×10^{-4}	24/30
0.7×10^{-4}	24/30
0.6×10^{-4}	26/30
0.5×10^{-4}	26/30

Note: Predictions based on Equations 1 and 2 in the text.

Methods

The empirical predictive model of Chou and Fasman (5) along with their latest conformational parameters (7) derived from 408 β-turns (6) was used to analyze for β-turns in the vicinity of phosphorylated sites. The relative probability of observing a β-turn is defined as (8)

$$p_t = f_i \times f_{i+1} \times f_{i+2} \times f_{i+3} \qquad \text{(Equation 1)}$$

where f_i, f_{i+1}, f_{i+2}, and f_{i+3} are the respective frequency of occurrence of each residue in the first, second, third, and fourth positions of the β-turn.

The average p_t value for a known β-turn was found to be $\langle p_t \rangle = 0.5 \times 10^{-4}$. This figure results in overprediction which is partially minimized by using a cut-off value of 1.5 times the average p_t value (i.e., 0.75×10^{-4}). In addition, the tetrapeptide must fulfill the following conditions:

$$\langle P_\alpha \rangle < \langle P_t \rangle > \langle P_\beta \rangle \text{ and } \langle P_t \rangle > 1.00 \qquad \text{(Equation 2)}$$

$\langle P_t \rangle$, $\langle P_\alpha \rangle$, and $\langle P_\beta \rangle$ values are the average of the normalized frequencies of residues of the tetrapeptide in the β-turn, α-helical, and β-sheet structures, respectively. Sequences of proteins used herein were extended beyond the phosphorylated sequences reported previously (2, 9, 10, 11) when available in the Atlas of Protein Sequences (12).

Results and Discussion

Using the evaluation scheme discussed above, twenty-four of the thirty phosphorylated residues are predicted as occurring within β-turns

Table 2

Phosphorylated Residues Occurring in Predicted β-turns

Protein	Predicted β-turn	p_t(x 10^{-4})	$\langle P_t \rangle$	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	Position of Phosphorylated Residues in turn	Sequence of Predicted turn	Reference
Histone H1	37-40	1.08	1.29	0.83	0.72	2nd	Ala-Ser(P)-Gly-Pro	2
Histone H1	102-105	1.68	1.27	0.88	0.77	4th	Ala-Ser-Gly-Ser(P)	2
Histone H1	160-163	2.47	1.24	0.92	0.70	1st	Ser(P)-Pro-Lys-Lys	2
Histone H2A	1-4	1.53	1.38	0.72	0.80	1st	Ser(P)-Gly-Arg-Gly	2
Histone H2A	16-19	1.21	1.08	0.84	0.91	4th	Thr-Arg-Ser-Ser(P)	2
Histone H2B	11-14	1.27	1.25	0.92	0.75	4th	Lys-Lys-Gly-Ser(P)	2
Histone H2B	31-34	0.91	1.08	0.97	0.84	2nd	Arg-Ser(P)-Arg-Lys	13
Histone H2B	35-38	0.94	1.19	0.94	0.84	2nd	Glu-Ser(P)-Tyr-Ser	2
Histone H4	1-4	1.53	1.34	0.72	0.80	1st	Ser(P)-Gly-Arg-Gly	2
Histone H5*	45-48	1.62	1.20	0.91	0.84	1st	Ser(P)-Ser-Arg-Gln	10
Histone H5*	88-91	1.56	1.30	0.83	0.77	4th	Gly-Ala-Gly-Ser(P)	10
Lysozyme†	21-24	0.75	1.27	0.75	0.98	4th	Arg-Gly-Tyr-Ser(P)	9
Muscle glycogen synthetase	3-6	0.82	1.13	0.92	1.05	1st	Ser(P)-Val-Asp-Thr	15
Myelin Basic Protein	10-13	1.41	1.24	0.88	0.76	3rd	His-Gly-Ser(P)-Lys	2
Myelin Basic Protein	54-57	2.56	1.39	0.77	0.75	2nd	Gly-Ser(P)-Gly-Lys	2
Phosphorylase b	14-17	0.87	1.11	0.85	1.03	1st	Ser(P)-Val-Arg-Gly	2
Phosphorylase Kinase β-subunit	**	1.96	1.34	0.77	0.80	4th	Arg-Ser-Gly-Ser(P)	13
Protamine I-1A	6-9	1.77	1.31	0.82	0.80	1st, 2nd, 3rd	Ser(P)-Ser(P)-Ser(P)-Arg	2
Protamine I-1A	21-24	1.07	1.07	0.93	0.89	1st	Ser(P)-Arg-Arg-Arg	2
Protein Phosphatase Inhibitor-1	**	1.63	1.24	0.73	0.81	3rd	Arg-Pro-Thr(P)-Pro	14
Pyruvate Dehydrogenase α-subunit	**	1.17	1.22	0.84	0.81	4th	His-Gly-His-Ser(P)	11
Troponin-I	111-114	1.13	1.09	0.94	0.99	4th	Val-Lys-Ser-Ser(P)	2
			Positional Occurrence:			8/24 1st=33%; 5/24 2nd=21% 3/24 3rd=13%; 8/24 4th=33%		

* (There is uncertainty as to the site of attachment. In this case positions 45 and 91 were assumed. If the site of attachment were instead 46 and 92 they would still both be predicted as occurring within a β-turn.)

† Phosphorylation reduced, carboxymethylated, maleylated lysozyme.

** (Sequence numbers were not available.)

p_t, $\langle P_t \rangle$, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values were arrived at as discussed in the text.

(80%). If the lower value, 0.5×10^{-4} (the average p_t value of known turns), is used as a cut-off along with the other criteria, twenty-six out of thirty are predicted as β-turns (87%) (Table 1). The first and last positions of the β-turn are those most frequently predicted as the site of attachment (Table 2). Those sites of phosphorylation which are not found within a

Table 3

Sequences Containing Phosphorylated Residues

Not Predicted within β-turns

Protein	Sequence Containing Phosphorylated Residue and Position of Attachment	p_t (x 10^{-4})	Predicted Turns Near Phosphorylated Residues	Position of Phosphorylated Residue in Relation to Nearest Turns (t=res. of turn)†	Ref.
Histone H2B	Pro-Ala-Lys-Ser(P)-Ala-Pro-Lys Ac (6)	0.59	2-5; 7-10	t+1; t-1	2
Histone H5	Ser-Arg-Gln-Ser(P)-Ile-Gln-Lys (49)	0.50	45-48	t+1	10
Myelin Basic Protein	Arg-Gly-Leu-Ser(P)-Leu-Ser-Arg (110)	0.32	106-109; 112-115	t+1; t-1	2
Phosphorylase Kinase α-subunit	Arg-Leu-Ser(P)-Ile-Ser-Thr (3)	0.40	5-8	t-2	2
Troponin-I ‡	Arg-Ala-Ile-Thr(P)-Ala-Arg-Arg (36)	0.55	30-33	t+3	2
Troponin-I ‡	Ser(P)-Ala-Asp-Met (143)	0.95*	--	--	2
Pyruvate Kinase‡ (Pig liver)	Leu-Arg-Arg-Ala-Ser(P)-Leu	<0.75	--	--	2
Pyruvate Kinase‡ (Rat liver)	Arg-Arg-Ala-Ala-Ser(P)-Val-Ala	<0.75	--	--	13

* This is not a β-turn as $\langle P_\alpha \rangle = 1.16 > \langle P_t \rangle = 1.04$.

(Note: β-turns as predicted in text.)

† t+1 = one residue after the turn; t-1 = one residue before the turn.

‡ Not within 2 residues of a β-turn.

predicted β-turn region do, in four out of eight cases, fall within two residues of an adjacent β-turn (Table 3). Two proteins, troponin-I and pyruvate kinase are each phosphorylated at two sites which are not associated with β-turns.

The location of phosphorylated sites in or adjacent to regions predicted as β-turns has been demonstrated herein. The fact that each of the proteins investigated had at least one site of attachment located within a predicted β-turn and that those sites not occurring in a β-turn were close to a predicted turn (with two exceptions) indicates that the β-turn may be the site recognized by phosphorylating enzymes. Traugh et al (16) have reported the isolation of three protein kinases from rabbit reticulocytes differing in their phosphorylating specificity. The possibility of different classes of kinases, each requiring the recognition of a β-turn but differing in the site of attachment within or near these turns, is thus not to be overlooked. The wide spectrum of proteins investigated here and the reports of Aubert et al. (17) and Beely (18, 19) that glycosylated residues also have a high frequency of occurrence in or around predicted β-turns argues for a more important role for β-turns than has previously been thought. X-ray diffraction data will be necessary to confirm these findings.

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