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Amino acid–anticodon binding specificity: rationale for a new class of therapeutic agent

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In this article a new class of anticancer and antiviral drugs is discussed. These new drugs consist of small di- and tri-peptides, designed to bind to single-stranded (ss) regions that are crucial for the expression of genes such as the c-myc oncogene in cancers and start sites (and other ss regions) of viral pathogenic genes. The components (i.e. the amino acids and the sequences they form) of these peptides could be dictated by the specific binding of amino acids to their ss anticodons in tRNA.

Cancer cell viability depends on the continued overexpression of the c-myc oncogene, and thus this gene is a target of opportunity for anticancer agents. Sharply reducing the overexpression of c-myc leads to the death of cancer cells. To achieve this end the following rationale is suggested: crucial regions of the c-myc promoters (to which activating proteins must bind for expression to occur) are single stranded and thus strongly resemble the anticodon loop of tRNA. It was found that amino acids chemically bind to their cognate tRNA anticodons. Regarding the ss regions of c-myc as a series of adjacent 'anticodons', di- and tri-peptides are proposed to be aligned to their cognate 'anticodons' in the proper order. For example, if the ss region of a promoter is hypothetically TTT-GGG-CCC, the tripeptide Lys-Pro-Gly could be expected to bind to it and deny access of the promoter to all activating proteins, thereby blocking c-myc expression and all the cancers dependent on such overexpression. Similarly, it is reported that in the initial phase of gene expression the start sites of the genes are single stranded (before and after and spanning the start site). Thus, invoking the amino acid cognate anticodon binding specificity (ACABS) principle as described above, a series of small peptides are suggested that could span the start sites of pathogenic viral genes (e.g. the oris region of herpes simplex virus (HSV)) to deny access of the gene to the transcription elements. This would inactivate the toxic effect of the virus and thereby constitute a promising approach to antiviral therapy, where the start sites (or other ss regions of pathogenic genes) have been sequenced.

The ACABS principle (for peptide–nucleic-acid interaction) enables us to focus on probable effective small peptides rather than having to screen a large number of randomly chosen small peptides to find probable anticancer and antiviral therapeutic agents.

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Introduction

After a study of the literature dealing with small peptides and their possible interactions with nucleic acids, a new class of pharmaceuticals is discussed in this article: small peptides as specific repressors of genes where the gene products are associated with various diseases, including cancer.

Based on theoretical considerations, Melcher [1] predicted that amino acids should interact specifically with their cognate anticodons in the single-stranded (ss) region comprising the loop of the tRNA structure. Confirmation of Melcher's prediction was reported by Jungck [2] and by Weber and Lacy [3]. Other suggestions that there is indeed a specific affinity between amino acids and their cognate tRNA anticodons was reported in the case of tryptophan [4], histidine [5] and isoleucine [6]. Evidence obtained by fluorescence [7], UV shifts [8] and NMR [9] strongly supported this principle [referred to in this article as anticodon cognate amino acid binding specificity (ACABS)].

Other reports also seemed to offer confirmation of the validity of ACABS. In other experiments, Balasubramanian [10] showed that, on the basis of model building, it is possible to align two amino acids bound to their anticodon sequences in ss nucleic acids when the anticodon sequences are adjacent to each other. Khan and Roe [11] constructed DNA that had the same base sequences as tRNA (i.e. they made 'tDNA'), and found that amino acids bound to 'tDNA' with the same specificities that they have in tRNA, thus demonstrating that factors applying to tRNA sequences also apply to DNA sequences; in other words, the tRNA 2′OH is not a determining factor in tRNA recognition of its cognate amino acids. Therefore it is possible, based on ACABS, that a nona-deoxynucleotide TTTGGGCCC should bind to its anticodon cognate tripeptide Lys-pro-gly.

Work dealing with the effects of amino acids and proteins on HIV-1 could be explained in terms of ACABS. It was reported by Andersson *et al.* [12] that the amino acid glycine amide was effective at destroying the activity of HIV-1. Although these investigators ascribed this finding to events outside the HIV-1 trans-activation response (TAR) region, it is possible to conceive of an explanation in terms of ACABS. It is known that the activity of HIV-1 depends on the maintenance of the structural integrity of the TAR stem–loop conformation [13]. Bernacchi *et al.* [14] reported that there is equilibrium between the normal stem–loop structure of TAR and an open conformation (i.e. fully denatured ssRNA), which is inactive. This implies that anything that disrupts the normal stem–loop conformation will disable the HIV-1 virus. If the HIV-1 TAR structure is observed, one finds two overlapping triplet sequences at the base of the stem in TAR (reading from 5′ to 3′): ACC and CCC, which happen to be the cognate anticodons for the amino acid glycine. Thus, glycine could interact with both anticodon triplets synergistically, interfering with their Watson–Crick binding and destabilizing the crucial stem of TAR. The equilibrium would then favor the non-base-paired inactive open conformation. Another series of studies, done using HIV-1, might also lend credence to the validity of ACABS. It was found that the arginine-rich Tat protein binds to the bulge region of HIV-1 [15]. The sequence of bases in positions 23–25 is UCU, which is in fact the anticodon cognate of arginine. Thus, the interaction of Tat with HIV-1 could be explained in terms of ACABS.

This means that, based on ACABS, amino acids, even as part of di- or tri-peptides, can interact with and bind to ss nucleic acids so as to interfere with interactions of these sequences with other molecules that might be crucial to the function of the gene containing these sequences. If the gene is a pathogenic gene in viruses, the virus could be disabled by interaction with that small peptide.

Application of ACABS to the inhibition of expression of genes crucial for disease processes

Are there any precedents for small peptides (i.e. di- and tri-peptides) actually serving as gene repressors? Answering this, there are indeed such instances reported showing that certain di- or tri-peptides have been found to inhibit expression of specific genes. Marugg *et al.* [16] and Guédon *et al.* [17] showed that two dipeptides, prolylleucine and leucylproline, suppressed the expression of a proteinase gene in a bacterium. Jia *et al.* [18] found that a tripeptide, tyroservatide, inhibits important genes in a cancer cell. Hickson *et al.* [19] showed that alanylglutamine prevents the expression of glutamine synthetase that would occur in the presence of a steroid. Yang *et al.* [20] found the same tripeptide inhibited expression of genes associated with hemorrhagic shock. Liu *et al.* [21] found that acetylglu-ser-gly inhibited some genes (while activating others) in a lymphoma cell line. Thus, there is ample evidence for the specific inhibition of gene expression (i.e. transcription) by certain small peptides. This literature offers encouragement for the concept that other small peptides, as outlined below, could have the same effect: inhibiting expression of pathogenic genes (i.e. genes that lead to disease upon expression) and thus constituting another option in the treatment of various diseases. Furthermore, the information given above offers confidence that such specific small peptides could be designed using the ACABS principle.

Applications of ACABS in the treatment of various diseases with specific examples given

Treatment of cancers with small peptides designed to block expression of the c-myc oncogene

One of the major approaches to cancer therapy is to target the c-myc oncogene – the overexpression of which is required for the viability of cancer cells. Excellent reviews on this subject have been presented by authors such as Hermeking [22], among others [23–25]. These authors stress that the c-myc oncogene in tumors is an attractive target for cancer therapy. One of the major roles of c-myc is to cause genetic instability in the developing cancer [26,27]. It is not within the scope of this article to recapitulate the points made in these papers. Instead, it is the aim to present a new approach for the treatment of cancer based on the use of specific small peptides (i.e. di- and tri-peptides) to block the transcription of the c-myc oncogene, and thus stop all cancers reliant on c-myc overexpression.

As indicated above, to apply the ACABS principle it is necessary that the gene has crucial regions of ss nucleic acid (i.e. resembling the ss anticodon of tRNA) that are vital to the expression of the gene. Such opportunities are available in the regions of c-myc that activating proteins must bind so that the transcription process can proceed. Such a region was reported by Kinniburgh and coworkers [28–30], who sequenced such a region. A part of this ssDNA region has the sequence (dividing the sequence into triplets and regarding these triplets as quasi 'anticodons'): GGA-GGG-TGG(A). On the basis of ACABS, the following peptide is suggested as being capable of binding specifically to this region and thus blocking access to the proteins needed for transcription: Ser-Pro-Pro. Shifting the reading frame by one base, the sequence GAG-GGT-GGA is obtained, for which the anticodon cognate tripeptide (using the ACABS principle) is Leu-Thr-Ser. These are therefore the two candidate peptides that might be effective in blocking expression of c-myc and thus stopping the cancers dependent on such expression

('candidate peptide' = peptide proposed as a probable antidisease drug). Bazar *et al.* [31] reported a sequence in another part of the c-myc promoter (to which activating proteins must bind for c-myc to be expressed) that they refer to as far upstream element (FUSE). This sequence is also ss in character and, thus, could possibly serve as a target of opportunity using the ACABS principle. The activating protein binds only to the ssDNA region of FUSE [32]. In the central portion of this ssDNA region of FUSE there is the sequence TTT-AGG-CCT, to which the tripeptide (again using the ACABS principle) Lys-Pro-Arg should bind. This is therefore another candidate peptide. The only difficulty that might be encountered at this point is the stability of these peptides in the presence of cellular aminopeptidases. However, these aminopeptidases are usually highly specific and they do not act against all peptides. It is encouraging that the peptides mentioned earlier in the works of Hickson, Jia, Yang and Liu remain stable enough to exert their effects *in vivo*.

As mentioned above, one of the major functions of c-myc is to introduce, when overexpressed, a genetic instability from which mutations in DNA can emerge, as shown by Taylor et al. [26] and by Wade and Wahl [27]. This suggests that overexpression of c-myc must be curtailed before these irreversible changes in DNA (e.g. in K-ras) can occur. This was pointed out by D'Cruz *et al.* [33], they found that blocking c-myc expression led to the reversal of breast cancer progression if the cell had not yet developed instability. However, if the cancer cells had already exhibited K-ras point mutations the mutations did not regress.

By contrast, it was found that even in the presence of mutations (i.e. in K-ras) it is possible to kill cancer cells by blocking expression of c-myc [34]. Thus, even if mutations are introduced into cells owing to the instability induced by overexpression of c-myc, cancer cells might still remain highly susceptible to agents designed to block expression of c-myc, as is proposed in this article. It was found that targeting c-myc not only affects genetic stability but also, if expression of c-myc is curtailed, that there is a collapse of the tumor microenvironment and interference with tumor vascularization [25].

It could also be pointed out at this stage that observing mutated DNA in cancers might not be the result of direct attack by the carcinogens on DNA but might instead reflect carcinogen-induced deregulation of c-myc leading to the genetic instability that leads to DNA mutations. The unlikelihood that changes in DNA observed in cancers are the result of direct attack by carcinogens on DNA was pointed out in a recent paper [35].

Dosage of anti-c-myc peptides could be an important issue. It is well known that c-myc expression is important for the proliferation and viability of normal cells. Therefore, could targeting c-myc as a means of stopping cancers kill normal cells as well? The works of Wickstrom *et al.* [36] and of Williams *et al.* [37] offer some assurances regarding this point. They found that it took much more (perhaps as much as three times as much) anti-c-myc antisense DNA to kill normal cells than it took to kill leukemia cells. Thus, it might be possible to choose a dosage of anti-c-myc peptides that should be sufficient to kill cancer cells (i.e. by sharply reducing c-myc expression) without harming normal cells (i.e. destroying c-myc expression altogether). Of course, it would require much further investigation to determine such optimum dosages.

As far as side effects of c-myc expression inhibition are concerned, the report by Soucek *et al.* [25] is reassuring. These authors report that the side effects of inhibiting c-myc expression on normal cells can be tolerated over extended periods of time.

From the above considerations, it might be possible to nominate certain specific small peptides as feasible and plausible anticancer agents: a new class of anticancer drugs.

Use of small peptides as antiviral agents

As pointed out above, the ACABS principle can be applied if the crucial regions of a gene promoter are in the ssDNA conformation, which enables extrapolation from a similar ss anticodon region of tRNA. To set the stage for transcription of these genes, it is necessary that the start site is unpaired, so that transcription elements (i.e. transcription factors, RNA polymerase, mRNA precursors, among others) can have access to the start site and thus begin the process of transcription. Such ssDNA has been found to occur in many gene start sites [38–40] in the early phase of gene expression, extending as much as from −8 to +9 relative to the start site. It is possible, therefore, that small peptides spanning the start site could bind to the start site of genes so as to compete favorably with mRNA precursors and RNA polymerase for access to the start site. These small peptides could be designed using the ACABS principle. The following viruses might be targets of such peptides using the ACABS principle.

Human papillomavirus

Lace [41] has sequenced a crucial region of the human papillomavirus (HPV) genome including the Sp1 site. The Sp1 site sequence reported is ACTAAGGGCGTAAC. The Sp1 site is vital for the activity of this virus, based on work with a similar virus, bovine papillomavirus (BPV), as reported by Spalholz *et al.* [42] who found that disruption of the Sp1 site destroyed viral activity. Sp1, found to be important in the connexin 26 gene expression, seems to be in the ssDNA conformation [43]. Other evidence for the ss structure of Sp1 was reported [44]. Therefore, if it is firmly established that Sp1 is, in fact, ssDNA and it might be possible to apply ACABS to bind peptides to the Sp1 site and thus stop the expression of a region of this virus vital for its pathogenic effects.

Conceiving the ss region of the Sp1 site as a series of anticodons generates AGG-GCG-TAA. The anticodon cognate tripeptide for this 9-mer DNA sequence is Pro-Arg-Leu (with the COOH at either end of the peptide). It is suggested that candidate peptides could be dipeptide degradation products of the tripeptide (i.e. Pro-Arg, Arg-Leu). It is possible to shift the reading frame by one or two bases and design, by using the ACABS principle, other possible candidate peptides that could be used to combat HPV and related cancers (i.e. of the cervix) caused by this virus. For example, reading from the left of the above sequence, ACT-AAG-GGC-CTA, the following peptide (or the dipeptide degradation products) is suggested: Ser-Leu-Ala-Tyr.

Human T-cell leukemia virus

The major site of the early promoter of human T-cell leukemia virus (HTLV-1) has been sequenced [45]. The sequence around the start site is GGAGGGG*GCTCGCA (where the start site is indicated by *). Starting at the GGG spanning the start site (which should be ss) we have a nonanucleotide GGG-GGC-TCG. Based on the ACABS principle, and consulting all available anticodon cognate amino acid charts, we have the anticodon cognate peptide Pro-Ala-Arg. Shifting the reading frame over by one base we have AGG-GG*G-CTC, for which ACABS suggests Pro-Pro-Glu as a possible binding agent. Thus, by using these candidate peptides, we might have agents that will block the expression of HTLV-1 and thus act against the disease which depends on expression of HTLV-1.

Herpes simplex virus

Transcription of the oris region is vital for the toxicity of herpes simplex virus (HSV). The sequences around the start site of oris were sequenced by McCormick *et al.* [46] and found to be TGG-CT*C-TTT. The ACABS principle suggests that a peptide Pro-Glu-Lys should bind to this

start site (which, again, should be ss and thus we can apply the ACABS principle) and block expression of active HSV. Another sequence important for HSV toxicity is the intected cell protein (ICP) protein-coding segment. Its sequence around the start site is GCC-AT*C-AGC [47]. Based on ACABS, the peptide Gly-Asp-Ala should be effective in inactivating this gene segment and stop the diseases caused by HSV.

General procedure to use ACABS to construct antiviral peptides

(i) Isolate the virus to a degree of high purity. Subsequently identify the particular genes in that virus that are responsible for the toxic effects of the virus and locate the start site for transcription at either the crucial gene or for the entire virus.

(ii) Sequence the crucial genes especially noting the sequences around the start site(s) in that gene or for the entire virus. Based on the considerations given in this article such start sites should be (in the presence of RNA polymerase) ss in character and thus vulnerable to interacting with anticodon cognate amino acids arranged in the proper order as di- or tripeptides (again regarding the ss nucleic acid sequence in the start site vicinity as a series of adjacent 'anticodon' triplets).

(iii) Consult any chart of anticodons and formulate the suggested peptides based on the ACABS principle as described above. Design peptides that could span the start site to cover this region maximally and thus prevent its access to transcription factors or to other elements of the mRNA synthesis system. Try to avoid if possible N-end prolines or leucines in formulating the candidate peptides because these N-end peptides might be susceptible to degradation by cellular aminopeptidases.

(iv) Test the candidate peptides and similar-sized peptides that are randomly constructed (as controls) against synthesized segments of nucleic acid corresponding to the transcription site of the virus or gene, to test for specificity, using the appropriate gels to determine interactions or binding.

(v) If the results of procedure iv are encouraging (i.e. there is significantly more binding with the candidate peptide and the gene sequence than with the control randomly constructed sequence made up of any amino acid combination) test the candidate peptide *in vitro* and finally *in vivo* using infected animals as test systems.

Concluding remarks

Here, an approach to antiviral therapy that does not involve vaccines is described. It might be much easier to construct peptides based on the ACABS rationale described in this article than to construct antiviral vaccines, which can fail owing to a variety of reasons – some of which were documented by Frey and Monu [48] and by Whiteside [49]. Furthermore, instead of screening a vast array of different dipeptides and tripeptides, this article presents a rationale that might permit us to focus on the few candidate peptides that are likely to be effective.

Until now, biomedical scientists have been concerned with reactions involving polypeptides and proteins. It might appear that peptides as small as dipeptides might also have interesting properties. Depending on the conditions, some small peptides might be involved as gene activators in the action of growth factors (including carcinogens [34] and steroids), whereas others, such as those suggested in this article, might suppress expression of pathogenic genes and thus constitute a new class of therapeutic drug.

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