

this receptor, *Gr21a*, was shown to be expressed only in CO₂-sensitive neurons^{7,8}. This suggested that *Gr21a* might be involved in CO₂ sensation. However, *Gr21a* alone was insufficient to confer sensitivity to CO₂ when it was expressed in other neurons, implying that an essential factor in the process was still missing.

Jones *et al.*³ reasoned that the missing partner might also be similar to gustatory receptors. They discovered that one such gene, *Gr63a*, is indeed expressed with *Gr21a* in CO₂-sensitive neurons. Moreover, when these two genes were expressed together in another antennal neuron (a conventional olfactory neuron), they conferred robust responses to CO₂ on that cell. However, neither gene alone was sufficient to produce CO₂ sensitivity.

Next, the authors genetically engineered flies that lacked the *Gr63a* gene. In these 'knockout' flies, the neurons that normally respond to CO₂ were completely unresponsive. And whereas fruitflies normally avoid CO₂, the knockout flies were indifferent to this odour. This state of affairs was reversed by adding back a *Gr63a* gene to the knockout flies, demonstrating that loss of this gene was indeed responsible for the sensory deficit.

Together, these results demonstrate that both *Gr21a* and *Gr63a* are required for CO₂ perception in *Drosophila*. The simplest scenario is that the two receptors form a complex that binds to CO₂. It is possible, however, that other molecules are also required. If so, these components must be present in conventional

olfactory neurons, because *Gr21a* and *Gr63a* were together sufficient to confer CO₂ sensitivity when expressed in an arbitrary olfactory neuron elsewhere in the antenna.

Another open question is whether this putative receptor complex actually binds to CO₂. In vertebrates, elevation of CO₂ excites neurons that modulate breathing rhythms, increasing respiration and helping to clear CO₂ from the blood. This response is not, however, mediated by a direct action of CO₂. Instead, the neurons involved are activated by changes in pH that are secondary to CO₂ elevation⁹. A similar process might be occurring in the *Drosophila* antenna. If the receptor complex does bind to CO₂ directly, it will be interesting to discover what this binding site looks like. Many cellular responses to gases are mediated by metalloproteins, suggesting that a metal cofactor might have a role in this complex.

Understanding how this receptor complex interacts with CO₂ should also shed light on the unusual response properties of CO₂-sensitive neurons in insects^{10,11}. Compared with conventional olfactory neurons, these neurons are unusually insensitive to the velocity of air flow around the antenna. They signal concentration steps independently of background CO₂ levels, and respond to CO₂ increases and decreases in a remarkably symmetric way. Their concentration–response function is also nearly linear at concentrations near the typical ambient level of CO₂. Considered as tiny chemical sensors, these neurons are wonders of natural engineering.

Finally, the discoveries reported by Jones *et al.*³ have the potential to contribute to disease prevention. The most dangerous animals on Earth are in fact mosquitoes — mosquito-borne diseases cause more than a million deaths annually around the world. And like other blood-sucking insects, mosquitoes use CO₂ to locate their hosts. Jones *et al.* show that the mosquito relatives of *Gr21a* and *Gr63a* are co-expressed in the mosquito maxillary palp, a structure known to be the locus of CO₂ sensation in these insects. If this molecular insight permits the design of novel mosquito deterrents, it could have a major impact on global health. ■

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BIOORGANIC CHEMISTRY

A sweet synthesis

Linda C. Hsieh-Wilson

Peptides and proteins with sugars attached have many desirable biological properties, but their chemical synthesis is a technical challenge. An ingenious take on an old idea might simplify things considerably.

Part of what distinguishes us from bacteria is that the proteins in our bodies are decorated with elaborate arrays of sugars. Protein glycosylation — the attachment of sugars to the amino-acid building-blocks of proteins — plays a crucial role in such diverse processes as protein folding, cell–cell communication and viral invasion of cells. Yet it is conspicuously absent in many simple, unicellular organisms. Understanding the roles of these sugars and how their complex, disparate structures modulate the activities of proteins has been a long-standing challenge. Reporting in the *Journal of the American Chemical Society*¹, Brik and colleagues bring us a step closer to this goal by devising a clever strategy for generating glycopeptides — short sequences of amino acids with sugars attached — that may one day permit the tailored synthesis of glycoproteins.

Glycopeptides and glycoproteins are notoriously difficult to obtain as pure compounds, because they are naturally expressed as inseparable mixtures of different structures (glycoforms) that bear various sugars. This complexity makes it difficult to study how any specific glycoform affects a protein's function, which in turn complicates efforts to generate protein-based medicines. Indeed, most therapeutic glycoproteins are sold as mixtures of glycoforms, the active components of which are often unknown. One approach to solving this problem is to use chemical synthesis to create single structures.

Brik and colleagues¹ have now developed a strategy for assembling glycopeptides using a process known as peptide ligation. In their method, one peptide is attached to another that incorporates a modified sugar. A unique

feature of this approach is that the sugar assists the process by positioning the two peptides in close proximity to each other. Traditional glycopeptide synthesis is cumbersome, requiring excesses of reagents to drive reactions to completion, and often producing low yields of the desired products. Furthermore, strategies involving 'protecting groups' have been necessary to mask reactive chemical groups that do not participate directly in the reaction sequence. These requirements increase the complexity and the cost of glycopeptide synthesis. But by actively engaging a sugar in the ligation process, Brik *et al.* demonstrate that a variety of glycopeptides can be made in just a few steps and in high yield, without the need for protecting groups.

The authors' strategy is a clever twist on a well-established method for peptide synthesis known as native chemical ligation². In this process, two peptide fragments are joined together to form a larger fragment via a two-step mechanism. The first step involves the transient formation of a thioester bond between the two fragments (Fig. 1a), mediated by a reactive sulphur atom on one of the fragments. The resulting intermediate then undergoes a rapid, spontaneous rearrangement to form a peptide bond. The net result is the direct connection of two peptide fragments to form a



50 YEARS AGO

Among the numerous well-known scientists who were born in 1857... Ronald Ross is widely known, for the story of his long and patient attempts to identify the carrier of malarial fever has often been written... An outstanding centenary of the present year is that of the birth of Heinrich Rudolf Hertz, the German physicist, who was the first to detect electromagnetic waves in free space and measure their velocity... Elwood Haynes (1857–1925) is another American who should be remembered this year. He discovered several important alloys, including tungsten chrome steel, and in 1919 filed a patent for stainless steel... The question of the inheritance of acquired characteristics has recently received much attention. An early worker in this field of research was the Danish botanist W. L. Johannsen (1857–1927). One of the founders of modern research in heredity, he introduced the terms 'pure line', as well as 'gene', 'genotype' and 'phenotype'.
From *Nature* 5 January 1957.

100 YEARS AGO

In a recent note attention was directed to the recent renewal of experiments with Count Zeppelin's latest airship on the Lake of Constance... The 1906 Zeppelin airship... is 11 metres high, and each of the two cars can hold four persons, besides having a separate motor. The author states that with both motors working simultaneously a speed of 15 metres per second, or 54 kilometres per hour, can be maintained for sixty hours with the quantity of benzene the machine will carry... The advantages of the Zeppelin airship are more or less counterbalanced by the present necessity of using a sheet of water for starting and landing. Apart from the uses of such a machine in warfare, its applications in time of peace to the meteorological survey of the atmosphere are contemplated.
From *Nature* 3 January 1907.

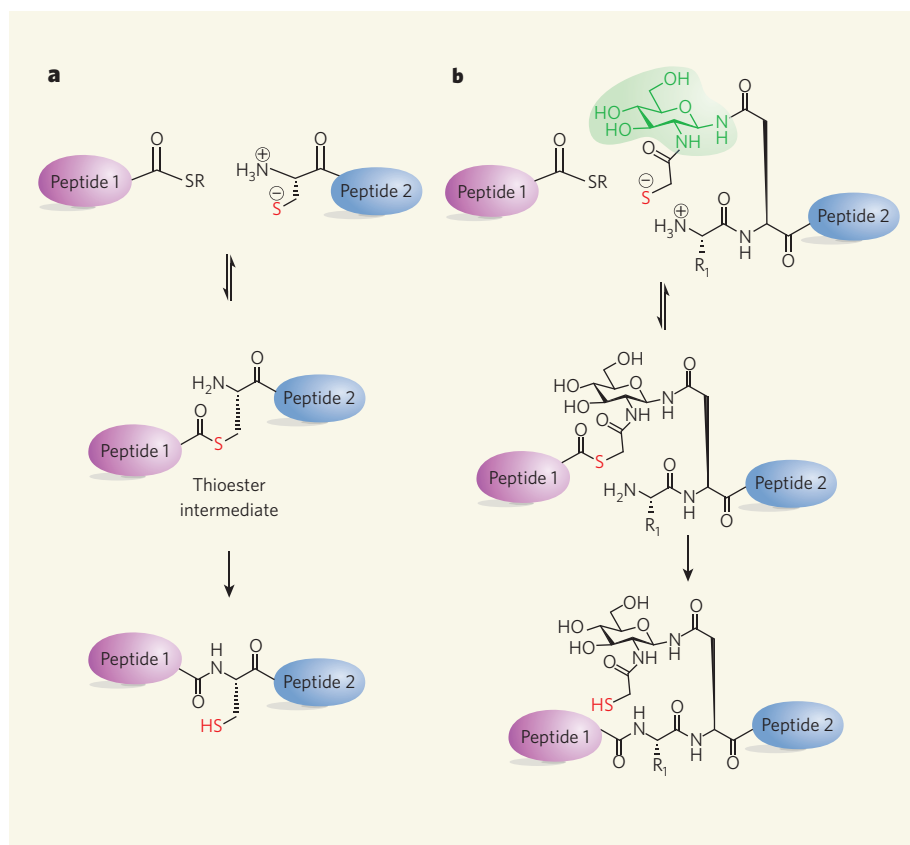


Figure 1 | Glycopeptide synthesis. **a**, Native chemical ligation is a well-established method for preparing peptides. A reactive sulphur atom (red) on the side-chain of a cysteine amino acid attacks another peptide (where R is typically a phenyl ring), producing a thioester intermediate that spontaneously rearranges to yield a peptide bond. **b**, Brik *et al.*¹ have modified this method to prepare glycopeptides, in which sugars are attached to peptide chains. A reactive sulphur atom (red) attached to an appended sugar (green) acts as a surrogate for the cysteine side-chain. Peptide bonds can thus be formed between a greater variety of amino acids. R₁ represents an amino-acid side-chain.

larger polypeptide. Moderately sized proteins have been produced in this way by sequential ligation of several peptide fragments, or through the coupling of a peptide to a larger protein fragment. Crucially, native chemical ligation provides exquisite control over the protein structure being formed, and allows the incorporation of various useful groups — such as synthetic amino acids, biophysical probes or stable isotopes of atoms used for structural studies — into selected sites within proteins^{3,4}.

Building on this approach, Brik and colleagues¹ attached a reactive sulphur group to a sugar within a peptide (Fig. 1b). In a process similar to the two-step mechanism for native chemical ligation, the authors reacted this sulphur group with a second peptide to form a thioester intermediate. This intermediate subsequently rearranges to give the desired product, in which the two starting materials are linked by a peptide bond. This strategy¹ has several remarkable features. Native chemical ligation requires cysteine — a sulphur-containing amino acid — to be at the reacting end of one of the peptides being joined together. By placing a reactive sulphur group on the sugar of a glycopeptide, rather than in an amino acid, the authors circumvent this requirement, thus

allowing bonds to be formed between a broader range of amino acids.

Moreover, a surprisingly wide array of amino acids is tolerated at the reaction site, thus permitting access to glycopeptides that are difficult to synthesize using other methods. Amino acids with small side-chains and those (such as histidine or aspartate) with side-chains that can serve as a base in the ligation pathway are favoured substrates in the reaction. Finally, the sulphur atom on the sugar provides a convenient handle for subsequent chemical manipulation — for example, it can be removed to give a naturally occurring sugar, reacted to append fluorescent dyes or other groups to the glycopeptide, or elaborated to form more complex sugars by using glycosyltransferase enzymes¹.

Further investigations are needed to assess the full scope of Brik and colleagues' reaction¹ and its potential application to glycoprotein synthesis. Nonetheless, the emergence of this and other methods^{5–8} for constructing pure peptides and proteins with sugars installed at preselected sites has many implications. For example, such techniques could transform the way therapeutic glycoproteins are discovered, developed and manufactured. Many of these proteins are obtained only as a mixture of glycoforms, just a fraction of which may

be biologically active⁹. But if drug-regulation authorities start to impose stringent regulations on glycoproteins (as they currently do for traditional 'small molecule' drugs, where the purity of the active form is paramount), then single glycoforms will be required. Furthermore, the ability to fine-tune the biological properties of therapeutic proteins by modifying their attached sugars could lead to exciting advances in drug discovery.

More fundamentally, having access to pure glycoproteins would help to elucidate the role of specific sugars in regulating protein structure and function. This could help to reveal how bacteria manage without these sweet appendages. Brik and colleagues' method¹ for making pure glycopeptides (and possibly glycoproteins) is truly a milestone achievement

in this rapidly developing field.

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DEVELOPMENTAL BIOLOGY

This worm is not for turning

Henry Gee

Molecular investigations of the origin of the dorso-ventral axis in an obscure marine invertebrate illuminate one of the longest-running debates in evolutionary biology — that over the origin of vertebrates.

Vertebrates are so different from other creatures that discovering their origins within the animal kingdom has always been problematic. But molecular, developmental and genomic work on the sometimes obscure invertebrate relatives of vertebrates is prompting a re-evaluation of this vexed topic.

As they recount in *PLoS Biology*, Lowe *et al.*¹ have been looking at the expression of genes associated with the specification of the dorso-ventral body axis — which surface becomes the upper (back) body surface and which the lower (belly) — in *Saccoglossus kowalevskii*, a worm-like member of the hemichordates. This is a group that is distantly related to the chordates, the larger group to which vertebrates themselves belong (Fig. 1). The authors find that the dorso-ventral axis in hemichordates is specified in a similar way to that in other animals. But this axis is decoupled from the development of the central nervous system — a later, chordate elaboration not found in hemichordates. This implies that the rules governing dorso-ventral axis formation are ancient and probably evolved with the first bilaterally symmetrical (bilaterian) multicellular animals.

The quest to understand the deployment of the dorso-ventral axis has been one of the most enduring themes in the study of vertebrate origins. It stems from the time of the wayward nineteenth-century savant Etienne Geoffroy Saint-Hilaire, who proposed that insects have the same basic body plan as vertebrates, only turned upside-down². This notion joined a list of seemingly eccentric theories about

vertebrate origins that has been lengthening ever since³. Another is the idea that vertebrates have independently invented a new kind of mouth on the opposite body surface to that in other animals. A third is that chordates and hemichordates evolved directly from ancestors akin to extinct, asymmetrical echinoderms (the group that includes modern sea-urchins

and starfishes), some of which seem to have sported the characteristic gill slits seen today in chordates and, as it happens, hemichordates.

Molecular work has disposed of most of these ideas — but not without highlighting valuable grains of truth in each of them. For example, construction of molecular evolutionary trees⁴ revitalized an old idea⁵ that echinoderms and hemichordates are sister taxa, in which case some primitive echinoderms really did have gill slits, no longer apparent in modern forms. Likewise, the discovery⁶ that insects have a genetic system of dorso-ventral specification similar to that of vertebrates — only inverted — gave Geoffroy Saint-Hilaire a new celebrity. Lowe *et al.*¹ build on this idea by showing that hemichordates exploit this same system in their development based on an axial polarity between two types of patterning molecule — BMP (bone morphogenetic protein) at one pole, and Chordin and its affiliates at the other. Baldly put, the dorso-ventral axis in all complex animals is determined largely by the antagonistic relationship of these two groups of agent.

In insects such as *Drosophila*, BMP is associated with what is conventionally regarded as the dorsal surface in the adult animal. In chordates, by contrast, BMP is a ventralizing agent. The inversion, however, is more apparent than real, having been determined after the fact by using the central nervous system — conventionally dorsal in chordates but ventral in insects — as a primary reference for telling which way is up.

Lowe and colleagues' work¹ on hemichordates adds welcome perspective. Because hemichordates have a diffuse nerve net rather than a central nervous system, this reference point disappears. Instead, we see that chordates differ from all other animals — hemichordates as well as insects — in the position of the mouth,

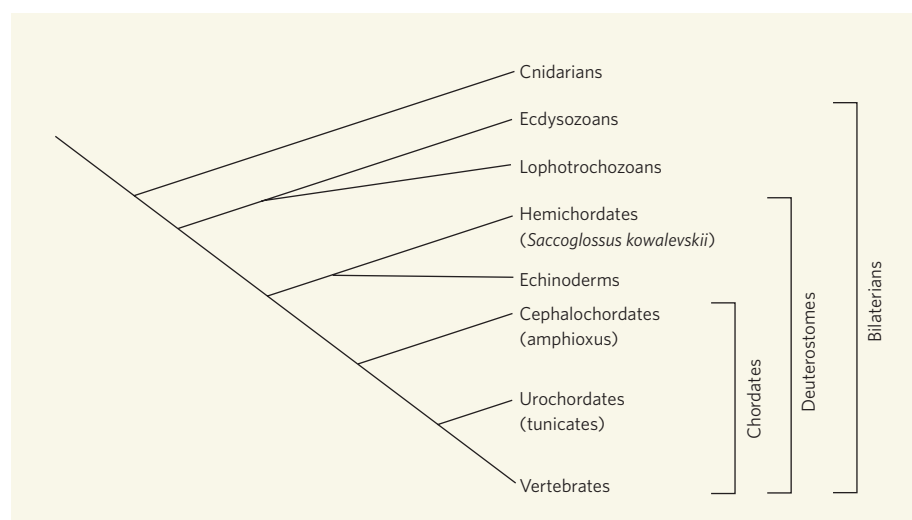


Figure 1 | Family connections. The relative position of the hemichordates in the evolutionary picture, and so of *Saccoglossus kowalevskii*, Lowe and colleagues' study subject¹. Hemichordates, along with echinoderms (sea-urchins and allies) and chordates (which include vertebrates), are the principal members of the deuterostomes, a much larger group within the bilaterians — the bilaterally symmetrical, multicellular animals. The other principal bilaterian groups of similar rank to the deuterostomes include the ecdysozoans (insects, nematodes and others) and the lophotrochozoans (molluscs, segmented worms and others). More primitive creatures such as cnidarians (jellyfishes and others) stand outside the bilaterian grouping.